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(54) Title: MORPHOGENIC PROTEIN-SPECIFIC CELL SURFACE RECEPTORS AND USES THEREFOR (57) Abstract Disclosed are: (1) nucleic acid sequences, amino acid sequences, homologies, structural features and various other data characterizing a morphogen cell surface receptors particularly OP-1-binding cell surface receptors; (2) methods for producing receptor proteins, including fragments thereof, using recombinant DNA technology; (3) methods for identifying novel morphogen receptors and their encoding DNAs; (4) methods and compositions for identifying compounds capable of modulating endogenous morphogen receptor levels; and (5) methods and compositions for identifying morphogen receptor binding analogs useful in the design of morphogen agonists and antagonists for therapeutic, diagnostic and experimental uses.		

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**"MORPHOGENIC PROTEIN-SPECIFIC CELL SURFACE RECEPTORS
AND USES THEREFOR"**

Field of the Invention

This invention relates generally to the field of tissue morphogenesis and more particularly to morphogenic protein-specific cell surface receptors.

5 Background of the Invention.

Cell differentiation is the central characteristic of tissue morphogenesis which initiates during embryogenesis, and continues to various degrees throughout the life of an organism in adult tissue repair and regeneration mechanisms. The degree of morphogenesis in adult tissue varies among different tissues and is related, among other things, to the degree of cell turnover in a given tissue.

The cellular and molecular events which govern the stimulus for differentiation of cells is an area of intensive research. In the medical and veterinary fields, it is anticipated that the discovery of the factor or factors which control cell differentiation and tissue morphogenesis will advance significantly medicine's ability to repair and regenerate diseased or damaged mammalian tissues and organs. Particularly useful areas for human and veterinary therapeutics include reconstructive surgery and in the treatment of tissue degenerative diseases including arthritis, emphysema, osteoporosis, cardiomyopathy, cirrhosis, degenerative nerve diseases, inflammatory diseases, and cancer, and in the regeneration of tissues, organs and limbs. (In this and related applications, the terms "morphogenetic" and "morphogenic" are used interchangeably.)

A number of different factors have been isolated in recent years which appear to play a role in cell differentiation. Recently, a distinct subfamily of the "superfamily" of structurally related proteins referred to in the art as the "transforming growth factor- β (TGF- β) superfamily of proteins" have been identified as true tissue morphogens.

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The members of this distinct "subfamily" of true tissue morphogenic proteins share substantial amino acid sequence homology within their morphogenetically active C-terminal domains (at least 50% identity in the C-terminal 102 amino acid sequence), including a conserved six or seven
5 cysteine skeleton, and share the in vivo activity of inducing tissue-specific morphogenesis in a variety of organs and tissues. The proteins apparently contact and interact with progenitor cells e.g., by binding suitable cell surface molecules, predisposing or otherwise stimulating the cells to proliferate and differentiate in a morphogenetically permissive environment.
10 These morphogenic proteins are capable of inducing the developmental cascade of cellular and molecular events that culminate in the formation of new organ-specific tissue, including any vascularization, connective tissue formation, and nerve innervation as required by the naturally occurring tissue. The proteins have been shown to induce morphogenesis of both bone cartilage and
15 bone, as well as periodontal tissues, dentin, liver, and neural tissue, including retinal tissue.

The true tissue morphogenic proteins identified to date include proteins originally identified as bone inductive proteins. These include OP-1, (osteogenic protein-1, also referred to in related applications as "OP1"),
20 its Drosophila homolog, 60A, with which it shares 69% identity in the C-terminal "seven cysteine" domain, and the related proteins OP-2 (also referred to in related applications as "OP2") and OP-3, both of which share approximately 70-75% identity with OP-1 in the C-terminal seven cysteine domain, as well as BMP5, BMP6 and its murine homolog, Vgr-1, all of which
25 share greater than 85% identity with OP-1 in the C-terminal seven cysteine domain, and the BMP6 Xenopus homolog, Vgl, which shares approximately 57% identity with OP-1 in the C-terminal seven cysteine domain. Other bone inductive proteins include the CBMP2 proteins (also referred to in the art as BMP2 and BMP4) and their Drosophila homolog, DPP. Another tissue morphogenic
30 protein is GDF-1 (from mouse). See, for example, PCT documents US92/01968 and US92/07358, the disclosures of which are incorporated herein by reference.

As stated above, these true tissue morphogenic proteins are recognized in the art as a distinct subfamily of proteins different from other members of the TGF- β superfamily in that they share a high degree of sequence identity in the C-terminal domain and in that the true tissue morphogenic proteins are

5 able to induce, on their own, the full cascade of events that result in formation of functional tissue rather than merely inducing formation of fibrotic (scar) tissue. Specifically, members of the family of morphogenic proteins are capable of all of the following in a morphogenetically permissive environment: stimulating cell proliferation and cell differentiation, and

10 supporting the growth and maintenance of differentiated cells. The morphogenic proteins apparently may act as endocrine, paracrine or autocrine factors.

The morphogenic proteins are capable of significant species "crosstalk." That is, xenogenic (foreign species) homologs of these proteins

15 can substitute for one another in functional activity. For example, DPP and 60A, two *Drosophila* proteins, can substitute for their mammalian homologs, BMP2/4 and OP-1, respectively, and induce endochondral bone formation at a non-bony site in a standard rat bone formation assay. Similarly, BMP2 has been shown to rescue a *dpp*⁻ mutation in *Drosophila*. In their native form,

20 however, the proteins appear to be tissue-specific, each protein typically being expressed in or provided to one or only a few tissues or, alternatively, expressed only at particular times during development. For example, GDF-1 appears to be expressed primarily in neural tissue, while OP-2 appears to be expressed at relatively high levels in early (e.g., 8-day) mouse embryos. The

25 endogenous morphogens may be synthesized by the cells on which they act, by neighboring cells, or by cells of a distant tissue, the secreted protein being transported to the cells to be acted on.

A particularly potent tissue morphogenic protein is OP-1. This protein, and its xenogenic homologs, are expressed in a number of tissues,

30 primarily in tissues of urogenital origin, as well as in bone, mammary and salivary gland tissue, reproductive tissues, and gastrointestinal tract

tissue. It is also expressed in different tissues during embryogenesis, its presence coincident with the onset of morphogenesis of that tissue.

The morphogenic protein signal transduction across a cell membrane appears to occur as a result of specific binding interaction with one or more cell surface receptors. Recent studies on cell surface receptor binding of various members of the TGF- β protein superfamily suggests that the ligands can mediate their activity by interaction with two different receptors, referred to as Type I and Type II receptors to form a hetero-complex. A cell surface bound beta-glycan also may enhance the binding interaction. The Type I and Type II receptors are both serine /threonine kinases, and share similar structures: an intracellular domain that consists essentially of the kinase, a short, extended hydrophobic sequence sufficient to span the membrane one time, and an extracellular domain characterized by a high concentration of conserved cysteines.

A number of Type II receptor sequences recently have been identified. These include "TGF- β II", a TGF- β Type II receptor (Lin et al. (1992) Cell 68:775-785); and numerous activin-binding receptors. See, for example, Mathews et al. (1991) Cell 65:973-982 and international patent application WO 92/20793, published November 26, 1992, disclosing the "ActR II" sequence; Attisano et al., (1992) Cell 68:97-108, disclosing the "ActR-IIB" sequence; and Legerski et al. (1992) Biochem Biophys. Res. Commun 183:672-679. A different Type II receptor shown to have affinity for activin is Atr-II (Childs et al. (1993) PNAS 90:9475-9479.) Two Type II receptors have been identified in C. elegans, the daf-1 gene, (Georgi et al. (1990) Cell 61:635-645), having no known ligand to date, and daf-4, which has been shown to bind BMP4, but not activin or TGF- β (Estevez, et al. (1993) Nature 365:644-649.)

Ten Dijke et al. disclose the cloning of six different Type I cell surface receptors from murine and human cDNA libraries. ((1993) Oncogene 8:2879-2887, and Science (1994) 264:101-104. These receptors, referenced as ALK-1 to ALK-6 ("activin receptor-like kinases"), share significant sequence identities (60-79%) and several have been identified as TGF- β binding (ALK-5) or activin binding (ALK-2, ALK-4) receptors. Xie et al. also report a

Drosophila Type I receptor encoded by the sax gene (Science (1994) 263:1756-1759). The authors suggest that the protein binds DPP.

To date, the Type I receptors with which the morphogenic proteins described herein interact on the cell surface have not yet been identified, and no Type II receptor has been described as having binding affinity for OP-1 and its related sequences. Identification of these cell surface molecules, with which the morphogens interact and through which they may mediate their biological effect, is anticipated to enhance elucidation of the molecular mechanism of tissue morphogenesis and to enable development of morphogen receptor binding "analogs", e.g., compounds (which may or may not be amino acid-based macromolecules) capable of mimicing the binding affinity of a morphogen for its receptor sufficiently to act either as a receptor binding agonist or antagonist. These "analogs" have particular utility in therapeutic, diagnostic and experimental research applications.

It is an object of this invention to provide nucleic acid molecules and amino acid sequences encoding morphogenic protein binding cell surface receptors, particularly OP-1-specific binding receptor sequences. Another object is to provide methods for identifying genes in a variety of species and/or tissues, and in a variety of nucleic acid libraries encoding morphogenic protein binding receptors, particularly receptors that bind OP-1. Still another object is to provide means for designing biosynthetic receptor-binding ligand analogs, particularly OP-1 analogs, and/or for identifying natural-occurring ligand analogs, including agonists and antagonists, using the receptor molecules described herein, and analogs thereof. Another object is to provide antagonists, including soluble receptor constructs comprising the extracellular ligand-binding domain, which can modulate the availability of OP1 for receptor binding in vivo. Another object is to provide means and compositions for competing with activin-receptor and BMP2/4-receptor interactions. Yet another object is to provide means and compositions for ligand affinity purification and for diagnostic detection and quantification of ligands in a body fluid using OP1-specific cell surface receptors and ligand-binding fragments thereof. Still another object is to provides means

and compositions for modulating the endogenous expression or concentration of these receptor molecules. Yet another object is to provide ligand-receptor complexes and analog sequences thereof, as well as antibodies capable of identifying and distinguishing the complex from its component proteins. Still
5 another object is to provide means and compositions for modulating a morphogenesis in a mammal. These and other objects and features of the invention will be apparent from the description, drawings and claims which follow.

Summary of the Invention

10 Type I and Type II cell surface receptor molecules capable of specific binding affinity with true tissue morphogenic proteins, particularly OP-1-related proteins, now have been identified. Accordingly, the invention provides ligand-receptor complexes comprising at least the ligand binding domain of these receptors and OP-1 or an OP-1 receptor-binding analog as the
15 ligand; means for identifying and/or designing useful OP-1 receptor-binding analogs and OP-1-binding- receptor analogs; and means for modulating the tissue morphogenesis capability of a cell.

The morphogen cell surface receptors useful in this invention are referred to in the art as Type I or Type II serine/threonine kinase receptors.
20 They share a conserved structure, including an extracellular, ligand-binding domain generally composed of about 100-130 amino acids (Type I receptors; up to about 196 amino acids for Type II receptors), a transmembrane domain sufficient to span a cellular membrane one time, and an intracellular (cytoplasmic) domain having serine/threonine kinase activity. The intact
25 receptor is a single polypeptide chain of about 500-550 amino acids and having an apparent molecular weight of about 50-55 kDa.

Of particular utility in the methods and compositions of the invention are the Type I cell surface receptors referenced herein and in the literature as, ALK-2, ALK-3 and ALK-6, whose nucleic acids and encoded amino acid
30 sequences are represented by the sequences in Seq. ID Nos. 3, 5 and 7 respectively, and which, as demonstrated herein below, have specific binding affinity for OP1 and OP1-related analogs. Accordingly, in one embodiment, the

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receptor sequences contemplated herein include OP-1 binding analogs of the ALK-2, ALK-3 and ALK-6 proteins described herein.

As used herein, ligand-receptor binding specificity is understood to mean a specific, saturable noncovalent interaction between the ligand and the receptor, and which is subject to competitive inhibition by a suitable competitor molecule. Preferred binding affinities (defined as the amount of ligand required to fill one-half (50%) of available receptor binding sites) are described herein by dissociation constant (K_d). In one embodiment, preferred binding affinities of the ligand-receptor complexes described herein have a K_d of less than $10^{-7}M$, preferably less than $5 \times 10^{-7}M$, more preferably less than $10^{-8}M$. In another preferred embodiment, the receptor molecules have little or no substantial binding affinity for TGF- β .

As used herein, an "OP1-specific receptor analog" is understood to mean a sequence variant of the ALK-2, ALK-3 or ALK-6 sequences which shares at least 40%, preferably at least 45%, and most preferably at least 50%, amino acid identity in the extracellular ligand binding domain with the sequence defined by residues 23-122 of Seq. ID No. 7 (ALK-6), and which has substantially the same binding affinity for OP1 as ALK-2, ALK-3 or ALK-6. ALK-6 and ALK-3 share 46% amino acid sequence identity in their ligand binding domains. Accordingly, in one preferred embodiment, the OP1-specific receptor analogs share at least 46% amino acid sequence identity with the extracellular, ligand binding domains of ALK-6 or ALK-3.

As will be appreciated by those having ordinary skill in the art, OP1-specific receptor analogs also can have binding affinity for other, related morphogenic proteins. As used herein, an OP1-specific receptor analog is understood to have substantially the same binding affinity for OP-1 as ALK-2, ALK-3 or ALK-6 if it can be competed successfully for OP-1 binding in a standard competition assay with a known OP-1 binding receptor, e.g., with ALK-2, ALK-3 or ALK-6. In one preferred embodiment, OP1-specific receptor analogs have a binding affinity for OP-1 defined by a dissociation constant of less than about $10^{-7}M$, preferably less than about $5 \times 10^{-7}M$ or $10^{-8}M$. It is

anticipated however, that analogs having lower binding affinities, e.g., on the order of 10^{-6} M also will be useful. For example, such analogs may be provided to an animal to modulate availability of serum-soluble OP1 for receptor binding in vivo. Similarly, where tight binding interaction is
5 desired, for example as part of a cancer therapy wherein the analog acts as a ligand-receptor antagonist, preferred binding affinities may be on the order of 5×10^{-8} M.

In another embodiment, the OP-1 binding receptor analogs contemplated by the invention include proteins encoded by nucleic acids which hybridize
10 with the DNA sequence encoding the extracellular, ligand binding domain of ALK-2, ALK-3 or ALK-6 under stringent hybridization conditions, and which have substantially the same OP-1 binding affinity as ALK-2, ALK-3 or ALK-6. As used herein, stringent hybridization conditions are as defined in the art, (see, for example, Molecular Cloning: A Laboratory Manual, Maniatis et al.,
15 eds. 2d.ed., Cold Spring Harbor Press, Cold Spring Harbor, 1989.) An exemplary set of conditions is defined as: hybridization in 40% formamide, 5 X SSPE, 5 X Denhardt's Solution, and 0.1% SDS at 37°C overnight, and washing in 0.1 X SSPE, 0.1% SDS at 50°C.

In still another embodiment, the OP-1 binding receptor analogs
20 contemplated by the invention include part or all of a serine/threonine kinase receptor encoded by a nucleic acid that can be amplified with one or more primers derived from ALK-1 (Seq. ID No. 1), ALK-2, ALK-3 or ALK-6 sequence in a standard PCR (polymerase chain reaction) amplification scheme. In particular, a primer or, most preferably, a pair of primers represented by any
25 of the sequences of SEQ ID Nos. 12-15 are envisioned to be particularly useful. Use of primer pairs (e.g., SEQ. ID No. 12/15; 13/15; 14/15) are described in WO94/11502 (PCT/GB93/02367).

Useful OP1-specific receptor analogs include xenogenic (foreign species) homologs of the murine and human ALK sequences described herein,
30 including those obtained from other mammalian species, as well as other, eukaryotic, non-mammalian xenogenic homologs. Also contemplated are biosynthetic constructs and naturally-occurring sequence variants of ALK-2,

ALK-3 and ALK-6, provided these molecules, in all cases, share the appropriate identity in the ligand binding domain, and bind OP-1 specifically as defined herein. In one embodiment, sequence variants include receptor analogs which have substantially the same binding affinity for OP1 as ALK-2, ALK-3 or ALK-6 and which are recognized by an antibody having binding specificity for ALK-2, ALK-3 or ALK-6.

In another embodiment the receptors and OP-1 binding receptor analogs contemplated herein provide the means by which a morphogen, e.g., OP-1, can mediate a cellular response. In one embodiment these receptors include ALK-2, ALK-3, or ALK-6, or sequence variants or OP-1 binding analogs thereof. In another embodiment, ALK-1, including sequence variants thereof is contemplated to participate in an OP-1 mediated cellular response.

OP1-specific receptor analogs may be used as OP1 antagonists. For example, a soluble form of a receptor, e.g., consisting essentially of only the extracellular ligand-binding domain, may be provided systemically to a mammal to bind to soluble ligand, effectively competing with ligand binding to a cell surface receptor, thereby modulating (reducing) the availability of free ligand in vivo for cell surface binding.

The true tissue morphogenic proteins contemplated as useful receptor ligands in the invention include OP-1 and OP-1 receptor-binding analogs. As used herein, an "OP-1 analog" or "OP-1 receptor-binding analog" is understood to include all molecules able to functionally substitute for OP-1 in Type I receptor binding, e.g., are able to successfully compete with OP-1 for receptor binding in a standard competition assay. In one embodiment, useful OP-1 receptor-binding analogs include molecules whose binding affinity is defined by a dissociation constant of less than about $5 \times 10^{-6} \text{M}$, preferably less than about 10^{-7}M or $5 \times 10^{-7} \text{M}$. As for the OP-specific receptor analogs above, both stronger and weaker binding affinities are contemplated to be useful in particular applications. In one preferred embodiment, these receptor-binding OP-1 analogs also bind OP-1 specific Type II serine/kinase receptors.

The OP-1 analogs contemplated herein, all of which mimic the binding activity of OP-1 or an OP-1-related protein sufficiently to act as a substitute for OP-1 in receptor binding, can act as OP-1 agonists, capable of mimicking OP-1 both in receptor binding and in inducing a transmembrane effect e.g., inducing threonine or serine-specific phosphorylation following binding. Alternatively, the OP-1 analog can act as an OP-1 antagonist, capable of mimicking OP-1 in receptor binding only, but unable to induce a transmembrane effect, thereby blocking the natural ligand from interacting with its receptor, for example. Useful applications for antagonists include their use as therapeutics to modulate uncontrolled differentiated tissue growth, such as occurs in malignant transformations such as in osteosarcomas or Paget's disease.

OP-1 analogs contemplated by the invention, can be amino acid-based, e.g., sequence variants of OP-1, or antibody-derived sequences capable of functionally mimicking OP-1 binding to an OP-1-specific receptor. Examples of such antibodies may include anti-idiotypic antibodies. In a specific embodiment, the anti-idiotypic antibody mimics OP1 both in receptor binding and in ability to induce a transmembrane effect. Alternatively, the OP-1 analogs can be composed in part or in whole of other chemical structures, e.g., the analogs can be comprised in part or in whole of nonproteinaceous molecules. In addition, the OP-1 analogs contemplated can be naturally sourced or synthetically produced.

As used herein, OP-1 related sequences include sequences sharing at least 60%, preferably greater than 65% or even 70% identity with the C-terminal 102 amino acid sequence of OP-1 as defined in Seq ID NO.7, and which are able to substitute for OP-1 in ligand binding to ALK-2, ALK-3 or ALK-6, (e.g., able to compete successfully with OP-1 for binding to one or more of these receptors.) OP-1 related sequences contemplated by the invention include xenogenic homologs (e.g., the Drosophila homolog 60A), and the related sequences referenced herein and in the literature as OP-2, OP-3, BMP5, BMP6 (and its xenogenic homolog Vgr-1.) OP-1 related sequences also include sequence variants encoded by a nucleic acid which hybridizes with a DNA

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sequence comprising the C-terminal 102 amino acids of Seq. ID No. 9 under stringent hybridization conditions and which can substitute for OP1 in an OP1-receptor binding assay. In another embodiment, an OP1 sequence variant includes a protein which can substitute for OP1 in a ligand-receptor binding assay and which is recognized by an antibody having binding specificity for OP1.

As used herein, "amino acid sequence homology" is understood to mean amino acid sequence similarity, and homologous sequences sharing identical or similar amino acids, where similar amino acids are conserved amino acids as defined by Dayoff et al., Atlas of Protein Sequence and Structure; vol.5, Suppl.3, pp.345-362 (M.O. Dayoff, ed., Nat'l BioMed. Research Fdn., Washington D.C. 1978.) Thus, a candidate sequence sharing 60% amino acid homology with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 60% of the amino acids in the candidate sequence are identical to the corresponding amino acid in the reference sequence, or constitute a conserved amino acid change thereto. "Amino acid sequence identity" is understood to require identical amino acids between two aligned sequences. Thus, a candidate sequence sharing 60% amino acid identity with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 60% of the amino acids in the candidate sequence are identical to the corresponding amino acid in the reference sequence.

As used herein, all receptor homologies and identities calculated use ALK-6 as the reference sequence, with the extracellular domain reference sequence constituting residues 23-122 of Seq. ID No.7; and the intracellular serine/threonine kinase domain reference sequence constituting residues 206-495 of Seq. ID No.7. Similarly, all OP-1 related protein homologies and identities use OP-1 as the reference sequence, with the C-terminal 102 amino acids described in Seq. ID No. constituting the seven cysteine domain.

Also as used herein, sequences are aligned for homology and identity calculations as follows: Sequences are aligned by eye to maximize sequence identity. Where receptor amino acid extracellular domain sequences are

compared, the alignment first maximizes alignment of the cysteines present in the two sequences, then modifies the alignment as necessary to maximize amino acid identity and similarity between the two sequences. Where amino acid intracellular domain sequences are compared, sequences are aligned to maximize alignment of conserved amino acids in the kinase domain, where conserved amino acids are those identified by boxes in Fig.3. The alignment then is modified as necessary to maximize amino acid identity and similarity. In all cases, internal gaps and amino acid insertions in the candidate sequence as aligned are ignored when making the homology/identity calculation. Exemplary alignments are illustrated in Figs. 2 and 3 where the amino acid sequences for the extracellular and intracellular domains, respectively are presented in single letter format. In the figures "gaps" created by sequence alignment are indicated by dashes.

In one aspect, the invention contemplates isolated ligand-receptor complexes comprising OP-1 or an OP-1 analog as the ligand in specific binding interaction with an OP-1 binding Type I receptor or receptor analog, as defined herein. In another aspect, the invention contemplates the ligand-receptor complex comprises part or all of an OP-1 binding Type II receptor. Type II receptors contemplated to be useful include Type II receptors defined in the literature (referenced hereinabove) as having binding specificity for activin or a bone morphogenic protein such as BMP-4. Such Type II receptors include daf4, ActRII and AtrII. In still another aspect, the ligand-receptor complex comprises both a Type I and a Type II receptor and OP1, or an OP1 analog as the ligand. In all complexes, the bound receptor can comprise just the extracellular, ligand binding domain, or can also include part or all of the transmembrane sequence, and/or the intracellular kinase domain. Similarly, the OP-1 ligand may comprise just the receptor binding sequence, longer sequences, including the mature dimeric species or any soluble form of the protein or protein analog.

The OP-1 and OP-1 analogs described herein can interact specifically with Type I and Type II receptors also known to interact with other morphogenic proteins (e.g., BMP2/BMP4) and activin. Thus invention also

contemplates the use of OP-1 and OP-1 receptor-binding analogs as competitors of specific BMP-receptor and activin-receptor interactions. As will be appreciated by those having ordinary skill in the art, these binding competitors may act as either agonists or antagonists (e.g., to inhibit an
5 activin or BMP-mediated cellular response).

In another aspect, the invention contemplates binding partners having specific binding affinity for an epitope on the ligand-receptor complex. In a preferred embodiment, the binding partner can discriminate between the complex and the uncomplexed ligand or receptor. In another embodiment, the binding
10 partner has little or no substantial binding affinity for the uncomplexed ligand or receptor. In another preferred embodiment, the binding partner is a binding protein, more preferably an antibody. These antibodies may be monoclonal or polyclonal, may be intact molecules or fragments thereof (e.g., Fab, Fab', (Fab)'₂), or may be biosynthetic derivatives, including, but not
15 limited to, for example, monoclonal fragments, such as single chain F_v fragments, referred to in the literature as sF_vs, BABs and SCAs, and chimeric monoclonals, in which portions of the monoclonals are humanized (excluding those portions involved in antigen recognition (e.g., complementarity determining regions, "CDRs".) See, for example, U.S. Pat. Nos. 5,091,513 and
20 5,132,405, the disclosures of which are incorporated herein by reference. Biosynthetic chimeras, fragments and other antibody derivatives may be synthesized using standard recombinant DNA methodology and/or automated chemical nucleic acid synthesis methodology well described in the art and as described below.

25 In still another aspect, the invention provides molecules useful in the design and/or identification of receptor-binding morphogenic protein analogs as described below, as well as kits and methods, e.g., screening assays, for identifying these analogs. The molecules useful in these assays can include part or all of the receptor sequence of SEQ ID NO. 3, 5 or 7, including amino
30 acid sequence variants and OP-1 binding analogs and amino acid sequence variants thereof.

As described above, sequence variants are contemplated to have substantially the same binding affinity for OP-1 as the receptors represented by the sequences in SEQ. ID Nos. 3-7. OP-1 binding receptor analogs include other, known or novel Type I or Type II serine/threonine kinase receptors having binding affinity and specificity for OP-1 as defined herein and which (1) share at least 40% amino acid identity with residues 23-122 of Seq. ID No. 7, (2) are encoded by a nucleic acid that hybridizes under stringent conditions with a nucleic acid comprising the sequence defined by nucleotides 256-552 of Seq ID No. 7; or (3) are encoded by a nucleic acid obtainable by amplification with one or more primer sequences defined by Seq. ID Nos. 12-15. Currently preferred for the assays of the invention are receptor sequences comprising at least the sequence which defines the extracellular, ligand binding domains of these proteins. The kits and assays may include just Type I receptors or both Type I and Type II receptors. Similarly, the kits and screening assays can be used in the design and/or identification of OP1-specific receptor analogs. The OP-1 receptor-binding analogs and OP-1-binding receptor analogs thus identified then can be produced in reasonable quantities using standard recombinant expression or chemical synthesis technology well known and characterized in the art. Alternatively, promising candidates can be modified using standard biological or chemical methodologies to, for example, enhance the binding affinity of the candidate analog as described in Example 10, below, and the preferred candidate derivative then produced in quantity.

In still another aspect, the receptor and/or OP1-specific receptor analogs can be used in standard methodologies for affinity purifying and/or quantifying OP1 and OP1 analogs. For example, the receptor's ligand binding domain first may be immobilized on a surface of a well or a chromatographic column; ligand in a sample fluid then may be provided to the receptor under conditions to allow specific binding; non-specific binding molecules then removed, e.g., by washing, and the bound ligand then selectively isolated and/or quantitated. Similarly, OP1 and OP1 analogs can be used for affinity purifying and/or quantifying OP1-specific receptors and receptor analogs. In

one embodiment, the method is useful in kits and assays for diagnostic purposes which detect the presence and/or concentration of OP1 protein or related morphogen in a body fluid sample including, without limitation, serum, peritoneal fluid, spinal fluid, and breast exudate. The kits and assays also
5 can be used for detecting and/or quantitating OP-1-specific receptors in a sample.

In still another aspect the invention comprises OP1-specific receptors and OP-1-binding receptor analogs useful in screening assays to identify organs, tissues and cell lines which express OP1-specific receptors. These
10 cells then can be used in screening assays to identify ligands that modulate endogenous morphogen receptor expression levels, including the density of receptors expressed on a cell surface. Useful assay methodologies may be modeled on those described in PCT US92/07359, and as described below.

The invention thus relates to compositions and methods for the use of
15 morphogen-specific receptor sequences in diagnostic, therapeutic and experimental procedures. Active receptors useful in the compositions and methods of this invention can include truncated or full length forms, as well as forms having varying glycosylation patterns. Active receptors useful in the invention also include chimeric constructs as described below. Active
20 OP1-specific receptors/analogues can be expressed from intact or truncated genomic or cDNA, or from synthetic DNAs in procaryotic or eucaryotic host cells, and purified, cleaved, refolded and oxidized as necessary to form active molecules. Useful host cells include prokaryotes, including E. coli and B. subtilis, and eukaryotic cells, including mammalian cells, such as
25 fibroblast 3T3 cells, CHO, COS, melanoma or BSC cells, Hela and other human cells, the insect/baculovirus system, as well as yeast and other microbial host cell systems.

Thus, in view of this disclosure, skilled genetic engineers now can, for example, identify and produce OP1-specific cell surface receptors or
30 analogs thereof; create and perform assays for screening candidate OP1 receptor-binding analogs and evaluate promising candidates and their progeny in therapeutic regimes and preclinical studies; modulate the availability of

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endogenous morphogen for cell surface interactions; modulate endogenous morphogen-specific cell surface receptor levels; elucidate the signal transduction pathway induced by morphogen-cell surface receptor binding; and modulate tissue morphogenesis in vivo.

5 Brief Description of the Drawings

Figure 1 is a schematic representation of the encoded ALK-2, ALK-3, ALK-6 amino acid sequences, showing the signal sequence 10, the transmembrane domain 12, the extracellular ligand binding domain 14, and the intracellular serine/threonine kinase domain 16;

10 Figure 2 is a homology alignment of the extracellular domains of ALK-2, ALK-3, and ALK-6, aligned to maximize amino acid identity, wherein conserved amino acids are identified by boxes and conserved cysteines are indicated by asterisks; and

Figure 3 is a homology alignment of the intracellular domain of ALK-2, 15 ALK-3 and ALK-6, aligned to maximize amino acid identity, wherein conserved amino acids are boxed and the serine/threonine kinase domain is indicated by arrows.

Detailed Description

Disclosed herein are Type I and Type II receptors having binding 20 specificity for true tissue morphogenic proteins, particularly OP1 and OP1-related proteins. It further has been determined that OP1 binds to a broader range of receptors than other known tissue morphogens or TGF- β family members. The Type I receptors disclosed herein, can be used together with OP1 and OP1 analogs for therapeutic, diagnostic and experimental uses as described herein 25 below. Moreover, soluble forms of the OP1-binding receptor proteins, e.g., forms consisting essentially of the extracellular domain or a fragment thereof sufficient to bind OP1 with specificity, can be used as a soluble therapeutic morphogen antagonist, as described below.

Following this disclosure, related OP1-specific receptors are 30 available, as are high and medium flux screening assays for identifying OP1 analogs and OP1-specific receptor analogs. These analogs can be naturally

occurring molecules, or they can be designed and biosynthetically created using a rational drug design and an established structure/function analysis. The analogs can be amino acid-based or can be composed in part or whole of non-proteinaceous synthetic organic molecules. Useful analogs also can

5 include antibodies, preferably monoclonal antibodies (including fragments thereof, e.g., Fab, Fab', and (Fab)'₂), or synthetic derivatives thereof, such as monoclonal single chain F_v fragments known in the art as sF_vs, BABs, and SCAs (see below), and bispecific antibodies or derivatives thereof. When these antibodies mimic the binding activity of OP-1 to a cell surface receptor

10 without inducing the biological response OP-1 does upon binding, the antibody can compete for OP-1 binding and act as an antagonist. These antibodies or derivatives thereof also can mimic OP-1 both in receptor binding and signal transduction, in which case the antibody acts as an OP-1 agonist. The antibodies and derivatives also can be used for inducing the morphogenic

15 cellular response by crosslinking receptors to morphogenic proteins, particularly OP1 and OP1-related proteins to form either homo- or hetero-complexes of the Type I and Type II receptors.

The OP1-binding receptor sequences described herein (ALK2, ALK3, ALK6) also can be used to create chimeric sequences, wherein, for example, part or

20 all of either the extracellular domain or the intracellular domain is a non-ALK sequence or is created from two or more ALK sequences. These chimeric receptors can be synthesized using standard recombinant DNA methodology and/or automated chemical nucleic acid synthesis methodology well described in the art and as disclosed below. Chimerics can be used, for example, in OP1

25 analog assays, wherein the OP1-binding extracellular domain is coupled to a non-ALK intracellular domain that is well characterized and/or readily detectable as a second messenger response system, as described below. Chimerics also can be used, for example, in high flux OP1 analogs screens and as part of purification protocols, wherein a soluble ligand binding domain of

30 an OP1-specific receptor is immobilized onto a support e.g., by covalent or non-covalent interactions, with a chromatographic matrix or the well surface of a 96-well plate. When immobilized onto a chromatographic matrix surface,

the receptor fragment can be used in a protocol to isolate OP1 or OP1 analogs. When immobilized on a well surface the receptor fragment is particularly useful in a screening assay to identify receptor-binding OP1 analogs in a standard competition assay.

5 The true tissue morphogenic proteins contemplated to be useful in the methods and compositions of the invention include forms having varying glycosylation patterns and varying N-termini. The proteins can be naturally occurring or biosynthetically derived, and can be produced by expression of recombinant DNA in prokaryotic or eukaryotic host cells. The proteins are
10 active as a single species (e.g., as homodimers), or combined as a mixed species. Useful sequences and eucaryotic and procaryotic expression systems are well described in the art. See, for example, US Patent Nos. 5,061,911 and 5,266,683 for useful expression systems.

 Particularly contemplated herein are OP1 and OP1-related sequences.
15 Useful OP1 sequences are recited in US Pat Nos. 5,011,691; 5,018,753 and 5,266,683; in Ozkaynak et al. (1990) EMBO J 9:2085-2093; and Sampath et al. (1993) PNAS 90: 6004-6008. OP-1 related sequences include xenogenic homologs, e.g.; 60A, from Drosophila, Wharton et al. (1991) PNAS 88:9214-9218; and proteins sharing greater than 60% identity with OP1 in the C-terminal seven
20 cysteine domain, preferably at least 65% identity. Examples of OP-1 related sequences include BMP5, BMP6 (and its species homolog Vgr-1, Lyons et al. (1989) PNAS 86:4554-4558), Celeste, et al. (1990) PNAS 87:9843-9847 and PCT international application WO93/00432; OP-2 (Ozkaynak et al. (1992) J.Biol. Chem. 267:13198-13205) and OP-3 (PCT international application WO94/06447).
25 As will be appreciated by those having ordinary skill in the art, chimeric constructs readily can be created using standard molecular biology and mutagenesis techniques combining various portions of different morphogenic protein sequences to create a novel sequence, and these forms of the protein also are contemplated herein.

30 A particularly preferred embodiment of the proteins contemplated by the invention includes proteins whose amino acid sequence in the cysteine-rich C-terminal domain has greater than 60% identity, and preferably greater than 65%

identity with the amino acid sequence of OPS (OP-1 sequence defining the C-terminal conserved six cysteines, e.g., residues 335-431 of Seq. ID No. 9).

In another preferred aspect, the invention contemplates osteogenic proteins comprising species of polypeptide chains having the generic amino acid sequence herein referred to as "OPX" which accommodates the homologies between the various identified species of the osteogenic OP1 and OP2 proteins, and which is described by the amino acid sequence presented below and in Sequence ID No. 11.

	Cys	Xaa	Xaa	His	Glu	Leu	Tyr	Val	Ser	Phe	
10	1				5					10	
	Xaa	Asp	Leu	Gly	Trp	Xaa	Asp	Trp	Xaa	Ile	
					15					20	
	Ala	Pro	Xaa	Gly	Tyr	Xaa	Ala	Tyr	Tyr	Cys	
					25					30	
15	Glu	Gly	Glu	Cys	Xaa	Phe	Pro	Leu	Xaa	Ser	
					35					40	
	Xaa	Met	Asn	Ala	Thr	Asn	His	Ala	Ile	Xaa	
					45					50	
	Gln	Xaa	Leu	Val	His	Xaa	Xaa	Xaa	Pro	Xaa	
20					55					60	
	Xaa	Val	Pro	Lys	Xaa	Cys	Cys	Ala	Pro	Thr	
					65					70	
	Xaa	Leu	Xaa	Ala	Xaa	Ser	Val	Leu	Tyr	Xaa	
					75					80	
25	Asp	Xaa	Ser	Xaa	Asn	Val	Ile	Leu	Xaa	Lys	
					85					90	
	Xaa	Arg	Asn	Met	Val	Val	Xaa	Ala	Cys	Gly	
					95					100	
30				Cys	His,						

and wherein Xaa at res. 2 = (Lys or Arg); Xaa at res. 3 = (Lys or Arg); Xaa at res. 11 = (Arg or Gln); Xaa at res. 16 = (Gln or Leu); Xaa at res. 19 = (Ile or Val); Xaa at res. 23 = (Glu or Gln); Xaa at res. 26 = (Ala or Ser); Xaa at res. 35 = (Ala or Ser); Xaa at res. 39 = (Asn or Asp); Xaa at res. 41 = (Tyr or Cys); Xaa at res. 50 = (Val or Leu); Xaa at res. 52 = (Ser or Thr); Xaa at res. 56 = (Phe or Leu); Xaa at res. 57 = (Ile or Met); Xaa at res. 58 = (Asn or Lys); Xaa at res. 60 = (Glu, Asp or Asn); Xaa at res. 61 = (Thr, Ala or Val); Xaa at res. 65 = (Pro or Ala); Xaa at res. 71 = (Gln or Lys); Xaa at res. 73 = (Asn or Ser); Xaa at res. 75 = (Ile or Thr); Xaa at res. 80 = (Phe or Tyr); Xaa at res. 82 = (Asp or Ser); Xaa at res. 84 = (Ser or Asn); Xaa at res. 89 = (Lys or Arg); Xaa at res. 91 = (Tyr or His); and Xaa at res. 97 = (Arg or Lys).

In still another preferred aspect, the invention contemplates osteogenic proteins encoded by nucleic acids which hybridize to DNA or RNA sequences encoding the C-terminal seven cysteine domain of OP1 or OP2 under stringent hybridization conditions.

- 5 A brief description of the various terms of OP-1 useful in the invention is described below:

OP1 - Refers generically to the family of osteogenically active proteins produced by expression of part or all of the hOP1 gene. Also referred to in related applications as "OPI" and "OP-1".

- 10 OP1-PP - Amino acid sequence of human OP1 protein (prepro form), Seq. ID No. 9, residues 1-431. Also referred to in related applications as "OP1-PP" and "OPP".

- OP1-18Ser - Amino acid sequence of mature human OP1 protein, Seq. ID No. 9, residues 293-431. N-terminal amino acid is serine. Originally identified as
15 migrating at 18 kDa on SDS-PAGE in COS cells. Depending on protein glycosylation pattern in different host cells, also migrates at 23kDa, 19kDa and 17kDa on SDS-PAGE. Also referred to in related applications as "OP1-18."

- OP1-16Ser; OP1-16Ala; OP1-16 Met; OP1-16 leu; OP1-16Val - N-terminally truncated mature human OP1 protein species defined, respectively, by residues
20 300-431; 316-431; 315-431; 313-431 and 318-431.

OPS - Amino acid sequence defining the C-terminal six cysteine domain, residues 335-431 of Seq. ID No. 9.

OP7 - Amino acid sequence defining the C-terminal seven cysteine domain, residues 330-431 of Seq. ID No. 9.

- 25 Soluble form OP1 - mature dimeric OP1 species having one or, preferably two copies of pro domain, e.g., at least residues 158-292 of Seq. ID No. 9, preferably residues 48-292 or 30-292, non-covalently complexed with the dimer.

The cloning procedure for obtaining OP1-binding ALK nucleic acid sequences, means for expressing receptor sequences, as well as other material aspects concerning the nature and utility of these sequences, including how to make and how to use the subject matter claimed, will be further understood from the following, which constitutes the best mode currently contemplated for practicing the invention.

Example 1. IDENTIFICATION OF ALK-1, ALK-2, ALK-3 and ALK-6

The cloning and characterization of ALK-1, -2, -3, and -6 receptors are described in detail in ten Dijke et al. (1993) Oncogene 8:2879-2887; and (1994) Science 264:101-104. The general structures of these proteins is described in Fig. 1, and the sequence alignments between the ALK genes are shown in Figs 2 and 3. These molecules have similar domain structures: an N-terminal predicted hydrophobic signal sequence (von Heijne (1986) Nucl. Acids Res. 14: 4683-4690) is followed by a relatively small extracellular cysteine-rich ligand binding domain, a single hydrophobic transmembrane region (Kyte & Doolittle (1982) J. Mol. Biol. 157, 105-132) and a C-terminal intracellular portion, which consists almost entirely of a kinase domain (Figure 3).

The extracellular domains of these receptors, defined essentially by residues 22-118 (SEQ. ID No. 1) for ALK-1; residues 16-123 (SEQ ID No. 3) for ALK-2; residues 24-152 (SEQ. ID No. 5) for ALK-3; and residues 23-122 (SEQ ID No. 7) for ALK-6, have cysteine-rich regions, but sequence similarity varies among the proteins. For example, ALK-3 and ALK-6 share a high degree of sequence similarity in their extracellular domains (46% identity) whereas ALK-2 shows less similarity with ALK 3 or ALK6 (see Fig. 2.)

The positions of many of the cysteine residues in these receptors can be aligned, indicating that the extracellular domains likely adopt a similar structural configuration.

The intracellular domains of these receptors are characterized by a serine/threonine kinase, defined essentially by residues 204-494 (SEQ. ID. No. 1) for ALK-1; residues 210-510 (SEQ ID No. 3) for ALK-2; residues 236-527 (SEQ ID No. 5) for ALK-3; and residues 206-497 (SEQ ID No. 7) for ALK-6. The catalytic domains of kinases can be divided into 12 subdomains with stretches

of conserved amino-acid residues. The key motifs are found in serine/threonine kinase receptors indicating that they are functional kinases. The consensus sequence for the binding of ATP (Gly-X-Gly-X-X-Gly in subdomain I followed by a Lys residue further downstream in subdomain II) is found in all the ALKS. Moreover, ALK-1, ALK-2, ALK-3 and ALK-6 have the sequence motifs or similar motifs HRDLKSKN (Subdomain VIB) and GTKRYMAPE (Subdomain VIII), that are found in most of the serine/threonine kinase receptors and can be used to distinguish them from tyrosine kinase receptors. Two short inserts in the kinase domain (between subdomain VIA and VIB and between X and XI are unique to members of this serine/threonine kinase receptor family. In the intracellular domain, these regions, together with the juxtamembrane part and C-terminal tail, are the most divergent between family members.

Type II serine/threonine kinase receptors known in the art are described and referenced herein above. Example 2. RECEPTOR EXPRESSION

15 A. General Considerations

Receptor DNA, or a synthetic form thereof, can be inserted, using conventional techniques well described in the art (see, for example, Maniatis (1989) Molecular Cloning A Laboratory Manual), into any of a variety of expression vectors and transfected into an appropriate host cell to produce recombinant protein polypeptide chains, including both full length and truncated forms thereof. Shortened sequences, for example, can be used for the production of soluble receptor fragments.

Useful host cells include E. coli, Saccharomyces cerevisiae, Pichia pastoris, the insect/baculovirus cell system, myeloma cells, and various other mammalian cells. The full length forms of the proteins of this invention preferably are expressed in mammalian cells, as disclosed herein. Soluble forms may be expressed from both mammalian or bacterial cell systems. The vector additionally may include various sequences to promote correct expression of the recombinant protein, including transcription promoter and termination sequences, enhancer sequences, preferred ribosome binding site sequences, preferred mRNA leader sequences, preferred protein processing sequences, preferred signal sequences for protein secretion, and the like.

The DNA sequence encoding the gene of interest also may be manipulated to remove potentially inhibiting sequences or to minimize unwanted secondary structure formation. The recombinant morphogen receptor also may be expressed as a fusion protein. After translation, the protein may be purified from the cells themselves or recovered from the culture medium. The DNA also may include sequences which aid in expression and/or purification of the recombinant protein. One useful sequence for example, is a hexa-His (His₆) sequence, which adds a histidine tail to allow affinity purification of the protein on an IMAC Cu²⁺ column (see below.)

10 For example, the DNA encoding the extracellular domain may be inserted into a suitable expression vector for transformation into a prokaryote host such as E. coli or B. subtilis, to produce a soluble, morphogen binding fragment. The DNA may expressed directly or may be expressed as part of a fusion protein having a readily cleavable fusion junction. An exemplary
15 protocol for prokaryote expression using MR-1 DNA is provided below. Recombinant protein is expressed in inclusion bodies and may be purified therefrom using the technology disclosed in U.S. Patent No. 5,013,653, for example.

 The DNA also may be expressed in a suitable mammalian host. Useful
20 hosts include fibroblast 3T3 cells, (e.g., NIH 3T3, from CRL 1658) COS (simian kidney ATCC, CRL-1650) or CHO (Chinese hamster ovary) cells (e.g., CHO-DXB11, from Lawrence Chasin, Proc. Nat'l. Acad. Sci. (1980) 77(7):4216-4222), mink-lung epithelial cells (MVLu), human foreskin fibroblast cells, human glioblastoma cells, and teratocarcinoma cells. Other useful eukaryotic cell
25 systems include yeast cells, the insect/baculovirus system or myeloma cells.

 To express an OP1-specific cell surface receptor, the DNA is subcloned into an insertion site of a suitable, commercially available vector along with suitable promoter/enhancer sequences and 3' termination sequences. Useful promoter/enhancer sequence combinations include the CMV promoter (human
30 cytomegalovirus (MIE) promoter) present, for example, on pCDM8, as well as the mammary tumor virus promoter (MMTV) boosted by the Rous sarcoma virus LTR enhancer sequence (e.g., from Clontech, Inc., Palo Alto). A useful inducible

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promoter includes, for example, A Zn^{2+} inducible promoter, such as the Zn^{2+} metallothionein promoter (Wrana et al. (1992) Cell 71:1003-1014.) Other inducible promoters are well known in the art and can be used with similar success. Expression also may be further enhanced using transactivating enhancer sequences. The plasmid also preferably contains an amplifiable marker, such as DHFR under suitable promoter control, e.g., SV40 early promoter (ATCC #37148). Transfection, cell culturing, gene amplification and protein expression conditions are standard conditions, well known in the art, such as are described, for example in Ausubel et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, NY (1989). Briefly, transfected cells are cultured in medium containing 5-10% dialyzed fetal calf serum (FCS), and stably transfected high expression cell lines obtained by amplification and subcloning and evaluated by standard Western and Northern blot analysis. Southern blots also can be used to assess the state of integrated receptor sequences and the extent of their copy number amplification.

The expressed protein then is purified using standard procedures. A currently preferred methodology uses an affinity column, such as a ligand affinity column or an antibody affinity column, the bound material then washed, and receptor molecules selectively eluted in a gradient of increasing ionic strength, changes in pH or addition of mild denaturants. Alternatively, where a useful anchor sequence has been added to the DNA, such as a $(His)_6$ sequence, the column may be a standard affinity column such as Cu^{2+} IMAC column. Here, for example, the cell culture media containing the recombinant protein is passed over a Cu^{2+} IMAC column (for example, prepared with 25 mM imidazole). The bound protein then is washed with a compatible solution and eluted with EDTA. The anchor sequence can be removed by a standard chemical or enzymatic procedure.

Mammalian cell expression is preferred where morphogen receptor expression on a cell surface is desired. For example, cell surface expression may be desired to test morphogen or morphogen analog binding specificity for a cell surface receptor under in vivo conditions. Cell surface expression also

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may be most efficacious for medium flux cellular screen assays as described below.

B.1 Exemplary Mammalian Cell Culture

The receptors tested in Examples 8 and 9 described below were expressed
5 in (1) COS-1 cells; (2) mink lung epithelial cells (Mv1Lu); (3) AG1518 human
foreskin fibroblasts; (4) MG-63 human osteosarcoma cells; (5) PC12 rat
pheochromocytoma cells (all obtained from American Type Culture Collection,
Rockville, MD); (6) U-1240 MG human glioblastoma cells (Bengt Westermark, et
al. (1988) Cancer Research 48:3910-3918); (7) Tera-2 teratocarcinoma cells
10 (clone 13, Thompson et al. (1984) J Cell Sci 72:37-64); (8) MC3T3-E1 cells
(Sudo et al. (1983) J. Cell Biol. 96:191-198, and (9) ROS 17/2.8 rat
osteosarcoma cells (Majeska et al. (1985) Endocrinology 116:170-179. The ROS
cells were cultured in Ham's F12 medium containing 14mM HEPES buffer, 2.5 mM
L-glutamine, 1.1 mM CaCl₂, 5% fetal bovine serum and antibiotics; MC3T3-E1
15 cells were cultured in a-MEM with 10% fetal bovine serum and antibiotics, and
Tera-2 cells were cultured in 5% CO₂ atmosphere at 37°C in a-MEM containing
10% fetal bovine serum, 100 units/ml of penicillin and 50 mg/ml of
streptomycin, using tissue culture dishes pretreated with 0.1% swine skin
gelatin (Sigma) in phosphate-buffered saline. Unless otherwise specified,
20 cells were cultured in DMEM containing 10% fetal bovine serum and antibiotics.

B.2 Transfection of cDNA

The receptors tested in Example 8.1 (ALK 1-6, daf-4) were transfected
as follows. Transient expression plasmids of ALK-1 to -6 and daf-4 were
generated by subcloning into an expression vector (pSV7d, Truett et al. (1985)
25 DNA 4:333-349) or into the pcDNA I expression vector (Invitrogen, San Diego).
For transient transfection, COS-1 cells were transfected with 10 mg each of
plasmids by a calcium phosphate precipitation method using a mammalian
transfection kit (Stratagene, La Jolla), following the manufacturer's
protocol. One day after transfection, the cells were used for the affinity
30 labeling and cross-linking experiments.

Example 3. ANTIBODY PRODUCTION

A. General Considerations

Antibodies capable of specifically binding the receptor molecules, ligand molecules, or the ligand-receptor complex itself, useful as analogs and useful in immunoassays and in the immunopurification of morphogen receptors described may be obtained as described below.

Where antibodies specific to the OP1-specific receptors are desired, but which do not interfere with ligand binding, the antigenic sequence preferably comprises the juxtamembrane sequence. Where antibodies capable of competing for ligand binding are desired, the ligand binding domain may be used as the antigen source. Where antibodies to the complex are desired, the complex itself preferably is used as the antigenic sequence and candidate antibodies then tested for cross reactivity with uncomplexed ligand and receptors versus the ligand-receptor complex. Finally, bispecific antibodies may used to complex ligand to a cell surface receptor (Type I or Type II) and/or to target an agent or ligand to cells or tissue expressing a Type I or Type II morphogen-specific receptor. Preferred bispecific antibody derived molecules are single chain binding sites described in US Pat No. 5,091,513 and 5,132,405, the disclosures of which are incorporated hereinabove by reference.

Antibodies useful as OP1 receptor-binding analogs may be obtained using the receptor ligand binding domain as the immunogen source and testing receptor-binding analogs for their ability to compete with OP1 in a competition binding assay. Similarly, where antibodies useful as OP1-specific receptor analogs are desired, OP1 is the immunogen source and the antibody candidate tested in a competition assay with receptor protein.

Polyclonal antibodies specific for a morphogen receptor of interest may be prepared generally as described below. Each rabbit is given a primary immunization (e.g., 500mg) of antigen in 0.1% SDS mixed with 500 ml Complete Freund's Adjuvant. The antigen is injected intradermally at multiple sites on the back and flanks of the animal. The rabbit is boosted after a month with 500mg of antigen in the same manner using incomplete Freund's Adjuvant. Test bleeds are taken from the ear vein seven days later. Two additional boosts and test bleeds are performed at monthly intervals until antibody against the

antigenic sequence is detected in the serum using a standard Western blot. Then, the rabbit is boosted monthly with 100 mg/ml of antigen and bled (15 ml per bleed) at days seven and ten after boosting.

Similarly, monoclonal antibodies specific for a given morphogen receptor molecule of interest may be prepared as described below: A mouse is given two injections of the antigenic sequence. The protein preferably is recombinantly produced. Where it is desired that the antibody recognize an epitope on the morphogen binding surface of a receptor an antigenic fragment derived from the extracellular domain preferably is provided. The first injection contains 100mg of antigen in complete Freund's adjuvant and is given subcutaneously. The second injection contains 50 mg of antigen in incomplete adjuvant and is given intraperitoneally. The mouse then receives a total of 230 mg of antigen in four intraperitoneal injections at various times over an eight month period. One week prior to fusion, the mouse is boosted intraperitoneally with antigen (e.g., 100 mg) and may be additionally boosted with an antigen-specific peptide conjugated to bovine serum albumin with a suitable crosslinking agent. This boost can be repeated five days (IP), four days (IP), three days (IP) and one day (IV) prior to fusion. The mouse spleen cells then are fused to commercially available myeloma cells at a ratio of 1:1 using PEG 1500 (Boehringer Mannheim, Germany), and the fused cells plated and screened for ALK-specific antibodies, e.g., using ALK-2, ALK-3 or ALK-6 as antigen. The cell fusion and monoclonal screening steps readily are performed according to standard procedures well described in standard texts widely available in the art. (See, for example, Guide to Protein Purification Murray P. Deutscher, ed., Academic Press, San Diego, 1990.

B. Exemplary ALK-Specific Antisera

Antibodies used in the assay of Example 8 were obtained as follows. Rabbit antisera against ALK-1 to -6 were made against synthetic peptides corresponding to the divergent, intracellular juxtamembrane parts. (ALK-1: residues 119-141; ALK-2: residues 151-172; ALK-3 residues 181-202; ALK-6: residues 151-168.) Peptides were synthesized with an Applied Biosystems 430 A Peptide Synthesizer using t-butoxycarbonyl chemistry, and purified by reverse

phase HPLC. The synthetic peptides were coupled to keyhole limpet hemocyanin (Calbiochem-Behring) using glutaraldehyde, as described by Gullick et al. (1985) EMBO J 4: 2869-2877. The coupled peptides then were mixed with Freund's adjuvant and used to immunize rabbits using standard methodologies.

5 Example 4. OP1-RECEPTOR BINDING ASSAYS

Ligand binding specificity is determined by evaluating the ability of a receptor molecule to bind a specific ligand, and the ability of that ligand to compete against itself and other molecules which bind the receptor. Useful ligands will have a binding affinity for a soluble morphogen receptor
10 extracellular domain such that dissociation constant (K_d) is less than about $10^{-6}M$, preferably less than $5 \times 10^{-7}M$. Where stronger binding interaction is desired, preferred affinities are defined by a K_d of 10^{-8} - $10^{-9}M$. OP1-related proteins are expected to be able to bind with specificity to multiple different receptor molecules, although likely with differing affinities.

15 Ligand binding specificity can be assayed as follows, essentially following standard protocols well described in the art and disclosed, for example, in Legerski et al. (1992) Biochem. Biophys. Res. Comm 183:672-679 and Frakar et al., (1978) Biochem. Biophys. Res. Comm. 80:849-857. In the ligand binding assays, a ligand having a known, quantifiable affinity for a morphogen
20 receptor molecule of interest is labelled, typically by radioiodination (^{125}I), e.g., by chromogenic or fluorogenic labeling, or by metabolic labelling, e.g., ^{35}S , and aliquots of cells expressing the receptor on their surface are incubated with the labelled ligand, in the presence of various concentrations of unlabelled potential competitor ligand. In the assays
25 described in Examples 8 and 9, below, this competitor typically is the candidate morphogen analog or an aliquot from a broth or extract anticipated to contain a candidate morphogen analog.

Alternatively, a crosslinking agent may be used to covalently link the ligand to the bound receptor, and the crosslinked complex then
30 immunoprecipitated with an antibody specific to the ligand, receptor, or complex. (See, Example 8.)

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A standard, exemplary protocol for determining binding affinity is provided below. Briefly, cells expressing a receptor on their cell surface are plated into 35 mm dishes and incubated for 48 hours in DMEM (Dulbecco's modified Eagle medium) plus 10% fetal calf serum. Purified morphogen, here, 5 e.g., OP-1, or an OP1-analog is iodinated with Na¹²⁵I by chloramine T oxidation, preferably having a specific activity of about 50-100 mCi/mg, essentially following the protocol of Frolik et al. (1984) J. Biol. Chem. 595:10995-11000. Labelled morphogen then is purified using standard procedures, e.g., chromatographically. Plated cells then are washed twice 10 with physiologically buffered saline in the presence of 0.1% BSA, and incubated at 22°C in the presence of BSA, buffer and labelled morphogen (1 ng) and various concentrations (e.g., 0-10mg/ml) of unlabelled competitor, e.g., unlabelled morphogen or candidate ligand analogs. Following binding, cells are washed three times with cold buffer, solubilized in 0.5 ml of 0.5 N NaOH, 15 removed from the dish, and radioactivity determined by gamma or scintillation counter. Data then are expressed as percent inhibition, where 100% inhibition of specific binding is the difference between binding in the absence of competitor and binding in the presence of a 100-fold molar excess of unlabelled morphogen. Binding parameters preferably are determined using a 20 computer program such as LIGAND (Munson et al. (1980) Anal. Biochem. 107:220-259.)

Where the receptor cell surface binding domain is to be provided as a soluble protein, the assay can be performed in solution, most readily as an immunoprecipitation assay. In currently preferred assays the morphogen 25 molecule is labelled and incubated with unlabelled receptor and candidate morphogen analogs. Receptor-specific antibody then is provided to the solution to precipitate the receptor-morphogen complex and the amount of labelled morphogen in the precipitated complex determined using standard detection means.

30 Where the receptor or ligand is to be used in an affinity isolation protocol, the molecule preferably is immobilized on a surface, preferably a matrix surface over which sample fluid will flow, allowing the ligand of

interest to bind, at letting nonbinding components pass through as effluent. The complex then can be removed intact or the ligand selectively removed with a desired eluant.

4.1 Screening Assay Considerations

5 In the analog screening assays described in Example 9 below, the preferred protocol for assaying ligand-receptor binding is a standard competition or radioimmunoassay (RIA). Here the OP1 is labelled and the relative binding affinity of a candidate OP1 analog ligand in a sample is measured by quantitating the ability of the candidate (unlabelled ligand
10 analog) to inhibit binding of the labelled ligand (competitor morphogen) by the receptor. In performing the assay, fixed concentrations of receptor and labelled morphogen are incubated in the absence and presence of unknown samples containing candidate ligands. Sensitivity can be increased by preincubating the receptor with candidate ligand before adding the labelled
15 morphogen. After the labelled competitor has been added, sufficient time is allowed for adequate competitor binding, and then free and bound labelled morphogen are separated, and one or the other is measured. Useful morphogen labels include radioactive labels, chromogenic or fluorogenic labels, and conjugated enzymes having high turnover numbers, such as horseradish
20 peroxidase, alkaline phosphatase, or b-galactosidase, used in combination with chemiluminescent or fluorogenic substrates. In the same manner, OP1-specific receptor analogs can be assayed for their affinity for OP1 in competition assays with labelled OP1 specific receptors.

Assays for evaluating a candidate OP1 receptor-binding analog's ability
25 to mimic OP-1 in signal-transduction across a membrane are exemplified in detail in Example 9.2, below. Briefly, the assay involves use of a cell (1) known to express an OP-1-specific receptor; or (2) which can be modified to express an OP-1-specific receptor, and/or (3) which can induce an OP-1-mediated cellular response. In the assay, the ability of a candidate analog
30 to induce an OP-1-specific cellular response is monitored. Numerous OP-1 responsive cells and OP-1-mediated inducible cellular and biochemical markers are known and described in the art. Alternatively, and as exemplified below,

an OP-1 inducible reporter gene system can be constructed and used to advantage in the assay.

4.2 Diagnostic Assay Considerations

The ability to detect morphogens in solution provides a valuable tool
5 for diagnostic assays, allowing one to monitor the level of morphogen free in the body, e.g., in serum and other body fluids. For example, OP-1 has been detected in a number of different body fluids, including serum and spinal fluid, including cerebro-spinal fluid, saliva, milk and other breast exudates. (See, for example, PCT US92/07432, PCT US93/07231, WO94/06449).

10 As one example, OP-1 is an intimate participant in normal bone growth and resorption. Thus, soluble OP-1 is expected to be detected at higher concentrations in individuals experiencing high bone formation, such as children, and at substantially lower levels in individuals with abnormally low rates of bone formation, such as patients with osteoporosis, aplastic bone
15 disease, or osteopenia. Monitoring the level of OP-1 in serum thus provides a means for evaluating the status of bone tissue and bone homeostasis in an individual, as well as a means for monitoring the efficacy of a treatment to regenerate damaged or lost bone tissue. Alternatively, the level of OP-1 in bone tissue can be assessed in a bone tissue biopsy.

20 Similarly, OP-1 and other morphogens have been identified in brain tissue. In particular, OP-1 is expressed and/or localized in developing and adult rat brain and spinal cord tissue, in the hippocampus, substantia nigra and the adenoma glial cells, as well as associated with astrocytes and the extracellular matrix surrounding neuronal cell bodies. (See, PCTUS93/07331).
25 Thus, monitoring the level of OP-1 in spinal fluid or associated with a nerve tissue biopsy can provide means for evaluating the status of nerve tissue in an individual, as well as means for monitoring the efficacy of a nerve regeneration or repair therapy.

For serum assays, the serum preferably first is partially purified to
30 remove some of the excess, contaminating serum proteins, such as serum albumin. Preferably the serum is extracted by precipitation in ammonium sulfate (e.g., 45%) such that the complex is precipitated. Further

purification can be achieved using purification strategies that take advantage of the differential solubility of soluble morphogen complex or mature morphogens relative to that of the other proteins present in serum. Further purification also can be achieved by chromatographic techniques well known in the art. The sample fluid then can be assayed for OP1 using the OP1-specific receptor(s) and binding assays as described herein.

For a tissue biopsy, cells can be collected and stained with a labelled OP-1-specific antibody or receptor molecule. Alternatively, the OP-1 protein selectively can be extracted and quantitated as described above.

Morphogens useful in the binding/screening assays contemplated herein include the soluble forms of the protein, e.g., the mature dimeric species complexed with one or two copies of the pro domain, the mature dimeric species alone, and truncated forms comprising essentially just the C-terminal active domain.

Example 5. TRANSMEMBRANE SIGNAL INDUCTION ASSAYS/OP1 MIMETICS

The kinase activity of the intracellular domains of the OP1-specific receptors can be tested in an autophosphorylation assay as described by Mathews et al. (PCT/US92/03825, published November 26, 1992). Briefly, the DNA fragment encoding at least the intracellular kinase domain of an OP1-specific receptor is subcloned into pGEX-2T (Smith et al. (1988) Gene 67:31-40) to create a fusion protein between the putative kinase domain and glutathione S-transferase (GST). The plasmid is introduced into E. coli and the expressed fusion protein purified using glutathione affinity chromatography. About 100-200 ng of fusion protein or purified GST then are incubated with 25 mCi (gp^{32}P) ATP in 50mM tris, 10mM MgCl_2 buffer for 30 minutes at 37° C. Products then are analyzed by gel electrophoresis and autoradiography. The fusion protein, but not GST alone, becomes phosphorylated, indicating that the kinase domain is functional. Phosphoamino acid analysis then can be performed to determine the predominant amino acid being phosphorylated. Similar assays can be performed using similar fusion constructs expressed in mammalian cells.

Various signaling transduction assays are provided in Example 9, below. An assay also can be developed for testing kinase activity transduction upon

ligand binding using a ligand-induced kinase activity assay known in the art. Here, the ability of OP-1 analog to induce phosphorylation upon binding to the receptor is tested.

See, for example, various assays for measuring ligand-induced kinase activity described by Accili et al. (1991) J. Biol.Chem. 266:434-439 and Nakamura et al. (1992) J.Biol. Chem. 267: 18924-18928,. For example, ligand-induced kinase activity (e.g., receptor autophosphorylation) can be measured in vitro by incubating purified receptor in the presence and absence of ligand (here, OP1 or OP1 analog, e.g., 10^{-7} M) under conditions sufficient to allow binding of the ligand to the receptor, followed by exposure to 32 P-ATP (e.g., 100mCi in the presence of 10mM Tris-HCl (pH 7.6), 10mM MgCl₂, 10mM MnCl₂, 1mM dithiothreitol, 0.15M NaCl₂, 0.1% Triton X-100 and 3% glycerol) and the amount of phosphorylation measured, e.g., by SDS polyacrylamide gel electrophoresis and autoradiography following immunoprecipitation with antiphosphoserine, antiphosphothreonine or antiphosphotyrosine antibody (e.g., commercially available or made using standard antibody methodologies.) While a low level of autophosphorylation may be detected in the absence of ligand, incubation with ligand is anticipated to significantly increase (e.g., 5-20 fold increase) the amount of phosphorylation detected.

In another assay for detecting ligand-induced receptor autophosphorylation, involving intact cells, receptor DNA is transfected into a suitable host cell, e.g., a fibroblast, which then is grown under standard conditions to create a confluent monolayer of cells expressing the receptor on their cell surface. On the day of the experiment, cells are incubated with or without ligand (e.g., OP1 or OP1 analog, e.g., 10^{-7} M) at 37°C, and then quickly washed with a "stopping solution" containing ATP (e.g., 0.1M NaF, 4 mM EDTA, 10mM ATP, 10 mM sodium orthovanadate, 4mM sodium pyrophosphate). The cells then are frozen in a dry ice/ethanol bath, solubilized and the receptors immunoprecipitated, e.g., with an antireceptor antibody, as described herein. The immune complexes then are segregated, washed, separated by gel electrophoresis using standard procedures and transferred to a membrane for

Western blot analysis using standard procedures. Phosphorylation of the receptor then can be visualized by immunodetection with a suitable antibody (e.g., antiphosphoserine, antiphosphothreonine or antiphosphotyrosine), as described above. The bound antibody (e.g., bound antiphosphoserine,

5 antiphosphothreonine or antiphosphotyrosine) then can be detected with ^{125}I labelled protein A, followed by autoradiography. The amount of phosphorylated receptor detected is anticipated to be significantly greater (5-20 fold increase) in receptors incubated with ligand than receptors exposed to ATP in the absence of ligand.

10 Ligand-induced receptor phosphorylation of exogenous substrates similarly can be assayed essentially using the methodology described herein. Here, a suitable substrate (e.g., a synthetic polypeptide containing serine, threonine or tyrosine amino acids) is provided to the receptor following ligand exposure and prior to incubation with ATP. The substrate subsequently
15 can be segregated by immunoprecipitation with an antibody specific for the substrate, and phosphorylation detected as described above. As for autophosphorylation, the amount of phosphorylated substrate detected following ligand incubation is anticipated to be greater than that detected for substrates exposed to receptors in the absence of ligand.

20 Alternatively, a reporter gene construct can be created to assay transmembrane signal induction. Here, the expression control elements for an OP-1 inducible protein marker is fused to the open reading frame sequence for any reporter gene, and induction of the reporter gene expression then assayed. Useful reporter genes include the luciferase gene or GAL4 as well as other,
25 easily characterizable markers.

Example 6. CHIMERIC RECEPTOR MOLECULES

Chimeric receptor molecules, e.g., comprising an ALK or ALK analog extracellular and transmembrane region and, for example, part or all of an intracellular domain from another, different receptor or an intracellular
30 domain from a different cell surface molecule, may be constructed using standard recombinant DNA technology and/or an automated DNA synthesizer to construct the desired sequence. As will be appreciated by persons skilled in

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the art, useful junctions include sequences within the transmembrane region and/or sequences at the junction of either the intracellular or the extracellular domains. Also envisioned are chimeres where the extracellular domain or the intracellular domains themselves are chimeric sequences.

5 Chimeric sequences are envisioned to be particularly useful in screening assays to determine candidate binding ligands (e.g., OP1 analogs, see below), where the non-receptor intracellular domain provides a suitable second messenger response system that is easy to detect. Potentially useful other second messenger response systems include those which, when activated,
10 induce phosphoinositide hydrolysis, adenylate cyclase, guanylate cyclase or ion channels.

Chimeric receptor molecules have particular utility in gene therapy protocols. For example, a population of cells expressing a chimeric morphogen receptor molecule on their surface and competent for expressing a desired
15 phenotype can be implanted in a mammal at a particular tissue locus. By careful choice of the ligand binding domain used on these receptors a physician can administer to the individual a morphogen agonist capable of: (1) binding to the chimeric receptor alone and (2) stimulating the proliferation and/or differentiation of the implanted cells without affecting
20 endogenous cell populations.

Example 7. CONSIDERATIONS FOR IDENTIFYING OTHER OP1 SPECIFIC RECEPTORS IN NUCLEIC ACID LIBRARIES

Identification of ALK Type I receptors that can bind OP-1 allows one to identify other morphogen receptor sequences in different species as well as
25 in different tissues. The OP1-binding ALK sequences themselves can be used as a probe or the sequence may be modified to account for other potential codon usage (e.g., human codon bias.) Currently preferred probe sequences are those which encode the receptor's extracellular domain.

Probes based on the nucleic acid sequence of Seq. ID Nos. 1, 3, 5 or 7
30 can be synthesized on commercially available DNA synthesizers, e.g. Applied Biosystems model 381A, using standard techniques, e.g. Gait, Oligonucleotide Synthesis: A Practical Approach, (IRL Press, Washington D.C., 1984). It is

preferable that the probes are at least 8-50 bases long, more preferably 18-30 bases long. Probes can be labeled in a variety of ways standard in the art, e.g. using radioactive, enzymatic or colormetric labels as described, for example, by Berent et al, (May/June 1985) Biotechniques: 208-220; and

5 Jablonski et al, (1986) Nucleic Acids Research 14: 6115-6128.

Preferably, low stringency conditions are employed when screening a library for morphogen receptor sequences using a probe derived from OP1-binding receptor. Preferred ALK-specific probes are those corresponding to bases encoding the extracellular domain ("ECD"), or encoding a unique
10 (nonhomologous) sequence within the cytoplasmic domain. Useful probes may be designed from bases encoding the juxtamembrane region, for example. The probe may be further modified to use a preferred species codon bias. Alternatively, probes derived from the serine/threonine kinase domain can be used to identify new members of the receptor kinase family which can be
15 screened for OP1 binding using the methods described in Example 8.

For example, for a probe of about 20-40 bases a typical prehybridization, hybridization, and wash protocol is as follows:

(1) prehybridization: incubate nitrocellulose filters containing the denatured target DNA for 3-4 hours at 55°C in 5X Denhardt's solution, 6X SSC (20X SSC
20 consists of 175g NaCl, 88.2 g sodium citrate in 800 ml H₂O adjusted to pH. 7.0 with 10N NaOH), 0.1% SDS, and 100 mg/ml denatured salmon sperm DNA,
(2) hybridization: incubate filters in prehybridization solution plus probe at 42°C for 14-48 hours, (3) wash; three 15 minutes washes in 6X SSC and 0.1% SDS at room temperature, followed by a final 1-1.5 minute wash in 6x SSC and 0.1%
25 SDS at 55°C. Other equivalent procedures, e.g. employing organic solvents such as formamide, are well known in the art.

Alternatively, morphogen receptor-specific DNA can be amplified using a standard PCR (polymerase chain reaction) methodology such as the one disclosed herein, to amplify approximately 500 base pair fragments. As for the
30 hybridization screening probes described above, the primer sequences preferably are derived from conserved sequences in the serine/threonine kinase

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domain. The primers disclosed herein, in Seq. ID Nos. 12-15 are envisioned to be particularly useful, particularly in combination..

Examples of useful PCR amplifications, including the use of the primers recited herein, are disclosed in ten Dijke, et al. (1993) Oncogene 8:2879-2887 and (1994) Science 264:101-104,, and which also describe the isolation protocols for ALK-1, ALK-2, ALK-3 and ALK-6.

7.1 TISSUE DISTRIBUTION OF MORPHOGEN RECEPTORS

Determining the tissue distribution of OP1-specific receptors can be used to identify tissue and cell sources which express these receptors, to identify new, related OP1-specific receptor molecules, as well as to identify target tissues for OP1-receptor interactions under naturally occurring conditions. The OP-1 specific receptor molecules (or their mRNA transcripts) readily are identified in different tissues using standard methodologies and minor modifications thereof in tissues where expression may be low. For example, protein distribution can be determined using standard Western blot analysis or immunohistological detection techniques, and antibodies specific to the morphogen receptor molecules of interest. Similarly, the distribution of morphogen receptor transcripts can be determined using standard Northern hybridization protocols and transcript-specific probes or by in situ hybridization.

Any probe capable of hybridizing specifically to a transcript, and distinguishing the transcript of interest from other related transcripts can be used. Because the morphogen receptors described herein likely share high sequence homology in their intracellular domains, the tissue distribution of a specific morphogen receptor transcript may best be determined using a probe specific for the extracellular domain of the molecule. For example, a particularly useful ALK-specific probe sequence is one derived from a unique portion of the 5' coding sequence, the sequence corresponding to the juxtamembrane region, or the 5' or 3' noncoding sequences. The chosen fragment then is labelled using standard means well known and described in the art and herein.

Using these receptor-specific probes, which can be synthetically engineered or obtained from cloned sequences, receptor transcripts can be identified and localized in various tissues of various organisms, using standard methodologies well known to those having ordinary skill in the art.

5 A detailed description of a suitable hybridization protocol is described in Ozkaynak, et al., (1991) Biochem. Biophys. Res. Commn. 179:116-123, and Ozkaynak, et al. (1992) J Biol. Chem. 267:25220-25227. Briefly, total RNA is prepared from various tissues (e.g., murine embryo and developing and adult liver, kidney, testis, heart, brain, thymus, stomach) by a standard

10 methodologies such as by the method of Chomczynski et al. ((1987) Anal. Biochem 162:156-159) and described below. Poly (A)+ RNA is prepared by using oligo (dT)-cellulose chromatography (e.g., Type 7, from Pharmacia LKB Biotechnology, Inc.). Poly (A)+ RNA (generally 15 mg) from each tissue is fractionated on a 1% agarose/formaldehyde gel and transferred onto a Nytran

15 membrane (Schleicher & Schuell). Following the transfer, the membrane is baked at 80°C and the RNA is cross-linked under UV light (generally 30 seconds at 1 mW/cm²). Prior to hybridization, the appropriate probe is denatured by heating. The hybridization is carried out in a lucite cylinder rotating in a roller bottle apparatus at approximately 1 rev/min for approximately 15 hours

20 at 37°C using a hybridization mix of 40% formamide, 5 x Denhardt's, 5 x SSPE, and 0.1% SDS. Following hybridization, the non-specific counts are washed off the filters in 0.1 x SSPE, 0.1% SDS at 50°C.

Example 8. Demonstration That ALK-2, ALK-3 and ALK-6
are OP1-Binding Receptors

25 The tissue morphogenic proteins OP1 and BMP4 were tested for specific binding interaction with the ALK receptors in receptor-transfected cells (where the receptor is over-expressed), and in nontransfected cells. It previously was known that ALK-5 interacted specifically with TGF β 1 and ALK-2 and ALK-4 interacted specifically with activin. In the experiment, complexes

30 were crosslinked and immuno-precipitated with an ALK-specific antibody as described below. To date, no binding with ALK-1 under the conditions of this protocol have been detected.

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Binding and affinity cross-linking using disuccinimidyl suberate (Pierce Chemical Co.) were performed using standard methods (e.g., Franzen et al. (1993) Cell 75:681-692 and Ichijo et al. (1990) Exp. Cell Res. 187:10995-11000.) A typical protocol is described below. Modifications from this

5 protocol for individual experiments were standard changes anticipated to produce the same result as for the recited procedure. Briefly, cells in multi-well plates were washed with binding buffer (e.g., phosphate buffered saline containing 0.9 mM CaCl_2 , 0.49mM MgCl_2 and 1 mg/ml bovine serum albumin (BSA)), incubated on ice in the same buffer with labelled ligand, in the

10 presence and absence of excess unlabelled ligand for sufficient time for the reaction to equilibrate (e.g., 3 hours.) Cells were washed and the crosslinking was done in the binding buffer without BSA together with 0.28 mM disuccinimidyl suberate for 15 min on ice. Cells were harvested by addition of 1 ml of detachment buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 10% glycerol,

15 0.3 mM PMSF.) Cells then were pelleted by centrifugation, then resuspended in 50 ml of solubilization buffer (125 mM NaCl, 10mM Tris-HCl, pH 7.4, 1mM EDTA, 1% Triton X-100, 0.3 mM PMSF, 1% Trasylol) and incubated for 40 minutes on ice. Cells were centrifuged again and supernatants subjected to analysis by standard SDS-gel electrophoresis using 4%-15% polyacrylamide gels, followed by

20 autoradiography.

Cell lysates obtained following affinity cross-linking via the general protocol described above were immunoprecipitated using antisera against ALKs (e.g., raised against the ALK juxtamembrane region), or directly analyzed by SDS-gel electrophoresis using gradient gels consisting of 5-12% or 5-10%

25 polyacrylamide. The gels were fixed and dried, and then subjected to autoradiography or analysis using phosphorImager (Molecular Dynamics).

8.1 Binding of OP-1 and/or BMP-4 to ALKs in Transfected Cells.

COS-1 cells transfected with ALK cDNA were tested for the binding of ^{125}I -OP-1 and ^{125}I -BMP-4, in the presence or absence of co-transfected Type II

30 receptor DNA: daf-4 cDNA or ActRII (Estevez et al. (1993) Nature 365:644-649 and Attisano et al. (1992) Cell 68:97-108, disclosing the DNA sequence for these Type II receptors and the disclosure of which is incorporated herein by

reference.) Since the cross-linked complexes were sometimes difficult to visualize because of high background, samples were immunoprecipitated by antisera against each ALK. The results are presented in Table I below. In the Table, "N/T" means "not tested". Binding was specific as determined by standard competition assays. The values represented by "+/-", "+", "++", "+++", and "-" are all qualitative descriptors of the relative amount of radioactivity measured when the crosslinked molecules were gel electrophoresed and subjected to autoradiography. More radioactivity measured indicates a stronger binding interaction detected. In the Table the strength of binding interaction is as follows: +++ > ++ > + > +/- > -.

TABLE I

	<u>¹²⁵I OP1</u>			<u>¹²⁵I BMP4</u>		
	<u>+daf4</u>	<u>-daf4</u>	<u>ActRII</u>	<u>+daf4</u>	<u>-daf4</u>	<u>+ActRII</u>
ALK1	-	-	-	ALK1	-	N/T
ALK2	++	+/-	++	ALK2	-	N/T
ALK3	++	-	+/-	ALK3	+++	++
ALK4	-	-	-	ALK4	-	N/T
ALK5	-	-	-	ALK5	-	N/T
ALK6	+++	++	+	ALK6	+++	+++

In the absence of daf-4, OP-1 bound to ALK-6, whereas BMP-4 bound to ALK-3 and ALK-6. Weaker binding of OP-1 to ALK-2 was also observed. Other ALKs did not bind OP-1 or BMP-4 in the absence of Daf-4. When ALK cDNAs were co-transfected with the daf-4 cDNA, increased binding of OP-1 to ALK-2 and ALK-6 was seen. In addition, ALK-3 also was found to bind OP-1 in the co-transfected cells. Similarly, increased binding of BMP-4 to ALK-3 and ALK-6 could be observed. Co-transfection of two different types of ALKs did not further increase the binding of OP-1 or BMP-4. In cells co-transfected with the DNA for ActRII and ALK-2, ALK-3 or ALK-6, OP1-receptor binding was enhanced.

The sizes of the cross-linked complexes were slightly higher for ALK-3 than for ALK-2 and ALK-6, consistent with its slightly larger size. Complexes

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of about 95 kDa as well as multiple components of 140-250 kDa were also co-immunoprecipitated with certain of the ALKs.

In standard competition assays performed with the Type I receptors in the presence and absence of the Type II receptors, the binding of OP-1 and BMP-4 could be competed with excess amounts of unlabeled OP-1, verifying the binding specificity of these interactions where they occurred.

These results demonstrate that ALK-2, ALK-3 and ALK-6 can serve as Type I receptors for OP-1. Notably, ligand binding apparently can be enhanced in the presence of Type II receptors. Moreover, OP-1 is able to interact with both a "bone morphogen" Type II receptor (daf 4) and an "activin" Type II receptor (ActRII), whereas, for example, activin only interacts with the ActRII Type II receptor. The data indicate that OP1 has a broader spectrum of receptor (Type I and Type II) binding affinities than do other tissue morphogenic proteins, or other members of the TGF- β family. It is anticipated that OP1 will have specific binding interactions with other "activin-binding" or "bone morphogen-binding" Type II receptors.

The ability of OP-1 to bind to Type I, Type II receptors having binding specificity for activin or BMP4 but not TGF-B, indicates that OP-1 and OP-1 analogs will be useful as competitors of activin or BMP4 binding to cell surface receptors. In particular, OP-1 and OP-1 analogs will be useful for competing with activin-ALK-2 binding and/or activin-ALK-2/ActRII (or other Type II) receptor binding; and for competing with BMP4 (or BMP2)-ALK-6 binding, and/or BMP2/4-ALK-6/daf 4 (or other Type II) binding. The OP-1 competitors may act as antagonists (e.g., binding competitors unable to induce the signal transduction cascade upon binding) or as agonists (e.g., able both to bind and induce the signal transduction cascade).

8.2 Identification of OP1-Specific Receptors in Nontransfected Cells

ALK-5 has been shown to bind TGF- β 1, and ALKs 2, 4 bind activin A with high affinities in nontransfected cells (ten Dijke, Oncogene, (1993); Science, (1994) referenced herein above.) In the present experiment, the binding affinity of OP1 and/or BMP4 to receptors in nontransfected cells was demonstrated as follows. The results corroborate the transfected cell data,

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verifying that OP1 interacts specifically with ALK-2, ALK-3 and ALK-6, but not ALK-4 or ALK-5.

MC3T3-E1 osteoblasts are well characterized cells known to respond to OP-1 and BMP-4 in the induction of alkaline phosphatase activity (Paralkar (1991) PNAS 8: 3397-3401.) These cells were affinity labeled using ^{125}I -OP-1 as described herein above, and cross-linked complexes of about 75 kDa were seen, which were immunoprecipitated only with the ALK-2 antiserum. Tera-2 teratocarcinoma cells and Mv1Lu cells responded to OP-1 as measured by production of plasminogen activator inhibitor-1 (PAI-1). Similar to MC3T3-E1 cells, cross-linked complexes using ^{125}I -OP-1 in Tera-2 teratocarcinoma were immunoprecipitated only by the ALK-2 antiserum.

On the other hand, cross-linked complexes using ^{125}I -OP-1 to Mv1Lu cells were immunoprecipitated by ALK-2 as well as ALK-3 and ALK-6 antisera. Mv1Lu cells are known to express ALK-4 and ALK-5 (Ebner (1993) Science 260:1344-1348), but cross-linked complexes with ^{125}I -OP-1 were not precipitated by antisera against these receptors. Similarly, cross-linked complexes in U-1240 MG glioblastoma cells were immunoprecipitated by ALK-2 and ALK-6 antisera, and weakly by ALK-3 antiserum. In contrast, cross-linking of ^{125}I -OP-1 to AG1518 human foreskin fibroblasts yielded weak immunoprecipitated components only by ALK-3 antiserum. Type II receptor-like components of about 95 kDa as well as high molecular weight complexes of 140-250 kDa co-immunoprecipitated with certain ALKs in the Tera-2 cells, Mv1Lu cells and U-1240 MG cells.

Receptors for BMP-4 have also been investigated using nontransfected cells. Cross-linked complexes using ^{125}I -BMP-4 to MC3T3-E1 cells and AG1518 human foreskin fibroblasts were immunoprecipitated only by ALK-3. On the other hand, cross-linking of ^{125}I -BMP-4 to Tera-2 cells did not yield any immunoprecipitated components by antisera against ALKs.

^{125}I -OP-1 and/or ^{125}I -BMP-4 also were demonstrated by affinity cross-linking to interact specifically with receptors in other BMP-responsive cells, e.g., MG-63 osteosarcoma cells and PC12 pheochromocytoma cells. A summary of

the binding of ALKs to OP-1 or BMP-4 in different cell types is shown in Table II, below. In the Table, "N/T" means not tested, and receptors presented in brackets indicate comparatively lower quantities of radioactive complexes detected.

TABLE II

<u>Cell lines</u>	<u>Binding of OP-1</u>	<u>Binding of BMP-4</u>
Mouse osteoblasts (MC3T3-E1)	ALK-2	ALK-3
Mink lung epithelial cells (Mv1Lu)	ALK-2, -3, -6	N/T
Human glioblastoma	ALK2, [-3], -6	N/T
Human teratocarcinoma (Tera-2)	ALK2	--
Human foreskin fibroblasts (AG1518)	[ALK3]	ALK3
Rat osteosarcoma (ROS17/2.8)	ALK2, [-3]	N/T.

5

Example 9. OP1, OP1-SPECIFIC RECEPTOR ANALOG SCREENING ASSAYS

The present invention is useful to determine whether a ligand, such as a known or putative drug, is capable of binding to and/or activating an OP1-specific cell surface receptor as described herein. Ligands capable of specific binding interaction with a given OP1-specific receptor (e.g., ALK-2, ALK-3, ALK-6) are referred to herein as OP1 analogs and can be used for therapeutic and diagnostic applications. Some analogs will have the ability to stimulate morphogenetic activity in the cell, mimicking both the receptor binding and signal transducing activity of OP1. These are referred to OP1 agonists or mimetics. Others will have strong binding affinity but will not stimulate morphogenesis, these are OP1 antagonists. The analogs can be amino acid-based, or they can be composed of non-proteinaceous chemical structures.

The methods and kits described below similarly can be used to identify OP1-specific receptor analogs, capable of mimicking the binding affinity of ALK-2, ALK-3 or ALK-6 for OP1. The analogs can be provided to a mammal to

20

interact with serum-soluble OP1, effectively sequestering the protein and modulating its availability for cell surface interaction.

Transfection of an isolated clone encoding a morphogen receptor into the cell systems described above provides an assay system for the ability of
5 ligands to bind to and/or to activate the receptor encoded by the isolated DNA molecule. Transfection systems, such as those described above, are useful as living cell cultures for competitive binding assays between known or candidate drugs and ligands which bind to the receptor and compete with the binding of
10 known morphogens, which are labeled by radioactive, enzymatic, spectroscopic or other reagents. Membrane preparations containing the receptor and isolated from transfected cells are also useful in these competitive binding assays. Alternatively, and currently preferred, purified receptor molecules or their ligand binding extracellular domains can be plated onto a microtiter well surface, in a modification of a sandwich assay, e.g., as a competition assay,
15 such as an RIA, described above. Finally, as described above, solution assays, and using only the receptor extracellular domain, also may be used to advantage in these assays. Functional assays of second messenger systems or their sequelae in transfection systems act as assays for binding affinity and efficacy in the activation of receptor function or efficacy in the antagonism
20 of receptor function. Such a transfection system constitutes a "drug discovery system", useful for the identification of natural or synthetic compounds with potential for drug development that can be further modified or used directly as therapeutic compounds to activate or inhibit the natural functions of the receptor encoded by the isolated DNA molecule.

25 Once such candidate drugs (e.g., OP-1 or receptor-binding analogs thereof) are identified, they can be produced in reasonable, useful quantities using standard methodologies known in the art. Amino acid-based molecules can be encoded by synthetic nucleic acid molecules, and expressed in a recombinant expression system as described herein above or in the art. Alternatively,
30 such molecules can be chemically synthesized, e.g., by means of an automated peptide synthesizer, for example. Non-amino acid-based molecules can be produced by standard organic chemical synthesis procedures. Where the

candidate molecule is of undetermined structure, or composition, its composition readily can be determined by, for example, mass spectroscopy. Two approaches to identifying analogs typically are practiced in the art: high flux screens and rational design of ligand mimetics. High flux screens typically screen naturally sourced materials or chemical banks for their ability to bind a protein of interest, here, e.g., the receptor. Typically, compounds are obtained from a range of sources, e.g., chemical banks, microbial broths, plant and animal extracts, and the like. In a high flux screen typically, purified receptor, preferably the soluble, ligand binding extracellular domain, is plated onto a microtiter well surface and a standard volume of a sample solution to be tested then is added. Also added is a standard volume having a known quantity of a purified ligand known to bind the receptor with specificity. Preferably the ligand is labelled with a substance that is readily detectable by automated means (e.g., radiolabel, chromophoric, fluorometric, enzymatic or spectroscopic label.) The wells then are washed and the amount of label remaining after washing or the amount of label remaining associated with the receptor then is detected. Positive scores are identified by the ability of the test substance to prevent interaction of the labelled ligand with the receptor. The screening assays can be performed without undue experimentation, using standard molecular and cell biology tools in common use in the art. For example, screening assays can be performed in standard 96-well plates. Fifteen such plates reasonably can be set up at a time to perform multiple screening assays in parallel. Thus, with only 10-11 reiterations of the screening assay 15,625 (5^6) compounds can be screened for their binding affinity. Even allowing for a maximum incubation time of 2 hours, all 15,625 compounds reasonably can be assayed in a matter of days.

High flux screens exploit both the high degree of specificity of the labelled ligand for its receptor, as well as high throughput capacity of computer driven robotics and computer handling of data. Candidate analogs identified in this manner, then can be analyzed structurally and this information used to design and to synthesize analogs having enhanced potency, increased duration of action, increased selectivity and reduced side effects.

Candidates also can be used in a rational design program as described below. Finally, candidate analogs also can be tested to determine morphogenetic effect, if any, as described below.

The second approach to the identification of analogs uses a rational design approach to create molecules capable of mimicking the binding effect of OP1 with an OP1-specific receptor. Here the relevant structure for receptor binding is analyzed to identify critical sequences and structures necessary for binding activity and this information can be used to design and synthesize minimal size morphogen analogs. As for candidate compounds in the high flux assay, design candidates can be tested for receptor binding activity as described above. As described above, a candidate sequence can be further modified by, for example standard biological or chemical mutagenesis techniques to create a candidate derivative having, for example, enhanced binding affinity or another preferred characteristic.

Antibodies capable of interacting specifically with the receptor and competing with OP1 binding also can be used as an analog. Antibodies can be generated as described above.

OP1 analogs may be evaluated for their ability to mimic OP1 or to inhibit OP1 binding (e.g., agonists or antagonists) by monitoring the effect of the analogs on cells bearing an OP1-specific receptor (e.g., ALK-2, ALK-3 or ALK-6.) OP1 agonists are anticipated to have utility in any application where tissue morphogenesis is desired, such as in the regeneration of damaged tissue resulting from mechanical or chemical trauma, degenerative diseases, tissue destruction resulting from chronic inflammation, cirrhosis, inflammatory diseases, cancer and the like, and in the regeneration of tissues, organs and limbs. OP1 antagonists are envisioned to have utility in applications where tissue morphogenesis is to be limited as, for example, in the treatment of malignant transformations including, but not limited to, osteosarcomas and Paget's disease.

Several exemplary systems for assaying the ability of a candidate analog transduce an OP-1-specific signal across the cellular membrane are described below.

9.1 Induction of Osteoblast Differentiation Markers

For example, OP1 is known to preferentially induce differentiation of progenitor cells, including embryonic mesenchymal cells and primary osteoblasts (see, for example, PCT US92/07432). As one example, OP1 analogs
5 can be tested for their ability to induce differentiation of primary osteoblasts, by measuring the ability of these analogs to induce production of alkaline phosphatase, PTH-mediated cAMP and osteocalcin, all of which are induced when primary osteoblasts are exposed to OP-1, 60A or DPP.

Briefly, the assays may be performed as follows. In this and all
10 examples involving osteoblast cultures, rat osteoblast-enriched primary cultures preferably are used. Although these cultures are heterogeneous in that the individual cells are at different stages of differentiation, these cultures are believed to more accurately reflect the metabolism and function of osteoblasts in vivo than osteoblast cultures obtained from established cell
15 lines. Unless otherwise indicated, all chemicals referenced are standard, commercially available reagents, readily available from a number of sources, including Sigma Chemical, Co., St. Louis; Calbiochem, Corp., San Diego and Aldrich Chemical Co., Milwaukee.

Rat osteoblast-enriched primary cultures are prepared by sequential
20 collagenase digestion of newborn suture-free rat calvaria (e.g., from 1-2 day-old animals, Long-Evans strain, Charles River Laboratories, Wilmington, MA), following standard procedures, such as are described, for example, in Wong et al., (1975) PNAS 72:3167-3171. Rat osteoblast single cell suspensions then are plated onto a multi-well plate (e.g., a 24 well plate) at a concentration
25 of 50,000 osteoblasts per well in alpha MEM (modified Eagle's medium, Gibco, Inc., Long Island) containing 10% FBS (fetal bovine serum), L-glutamine and penicillin/streptomycin. The cells are incubated for 24 hours at 37°C, at which time the growth medium is replaced with alpha MEM containing 1% FBS and the cells incubated for an additional 24 hours so that the cells are in serum-
30 deprived growth medium at the time of the experiment.

Alkaline Phosphatase Induction of Osteoblasts

The cultured cells in serum-free medium are incubated with OP1, OP1 analog or a negative control, using a range of concentrations. For example, 0.1, 1.0, 10.0, 40.0 or 80.0 ng OP-1/ml medium typically are used. 72 hours after the incubation period the cell layer is extracted with 0.5 ml of 1% Triton X-100. The resultant cell extract then, is centrifuged, and 100 µl of the extract is added to 90 µl of paranitrosophenylphosphate (PNPP)/glycerine mixture and incubated for 30 minutes in a 37°C water bath and the reaction stopped with 100 µl NaOH. The samples then are run through a plate reader (e.g., Dynatech MR700 plate reader, and absorbance measured at 400 nm, using p-nitrophenol as a standard) to determine the presence and amount of alkaline phosphate activity. Protein concentrations are determined by the Biorad method. Alkaline phosphatase activity is calculated in units/mg protein, where 1 unit=1 nmol p-nitrophenol liberated/30 minutes at 37°C. OP-1 induces a five -fold increase in the specific activity of alkaline phosphate by this method. Agonists are expected to have similar induction effects. Antagonists should inhibit or otherwise interfere with OP1 binding, and diminished alkaline phosphatase induction should result when the assay is performed with an antagonist in the presence of a limiting amount of OP1.

Induction of PTH-Mediated cAMP.

The effect of a morphogen analog on parathyroid hormone-mediated cAMP production in rat osteoblasts in vitro may be demonstrated as follows.

Rat osteoblasts are prepared and cultured in a multiwell plate as described above. The cultured cells then are divided into three groups: (1) wells which receive, for example, 1.0, 10.0 and 40.0 ng OP-1/ml medium); (2) wells which receive the candidate analog at various concentration ranges; and (3) a control group which receives no additional factors. The plate is then incubated for another 72 hours. At the end of the 72 hours the cells are treated with medium containing 0.5% bovine serum albumin (BSA) and 1mM 3-isobutyl-1-methylxanthine for 20 minutes followed by the addition into half of the wells of human recombinant parathyroid hormone (hPTH, Sigma, St. Louis) at a concentration of 200 ng/ml for 10 minutes. The cell layer then is extracted from each well with 0.5 ml of 1% Triton X-100. The cAMP levels then are

determined using a radioimmunoassay kit (e.g., Amersham, Arlington Heights, Illinois). OP-1 doubles cAMP production in the presence of PTH. Agonists are expected to have similar induction effects. Antagonists are expected to inhibit or otherwise interfere with OP1 binding, and diminished cAMP

- 5 production should result when the assay is performed with an antagonist in the presence of limiting the amount of OP1.

Induction of Osteocalcin Production

Osteocalcin is a bone-specific protein synthesized by osteoblasts which plays an integral role in the rate of bone mineralization in vivo.

- 10 Circulating levels of osteocalcin in serum are used as a marker for osteoblast activity and bone formation in vivo. Induction of osteocalcin synthesis in osteoblast-enriched cultures can be used to demonstrate morphogenic efficacy in vitro.

- Rat osteoblasts are prepared and cultured in a multi-well plate as
15 above. In this experiment the medium is supplemented with 10% FBS, and on day 2, cells are fed with fresh medium supplemented with fresh 10 mM b-glycerophosphate (Sigma, Inc.). Beginning on day 5 and twice weekly thereafter, cells are fed with a complete mineralization medium containing all of the above components plus fresh L(+)-ascorbate, at a final concentration of
20 50mg/ml medium. OP1 or OP1 analog then is added to the wells directly, e.g., in 50% acetonitrile (or 50% ethanol) containing 0.1% trifluoroacetic acid (TFA), at no more than 5ml OP1/ml medium. Control wells receive solvent vehicle only. The cells then are re-fed and the conditioned medium sample diluted 1:1 in standard radioimmunoassay buffer containing standard protease
25 inhibitors and stored at -20°C until assayed for osteocalcin. Osteocalcin synthesis is measured by standard radioimmunoassay using a commercially available osteocalcin-specific antibody and can be confirmed by Northern blot analysis to calculate the amount of osteocalcin mRNA produced in the presence and absence of OP-1 or OP1 analog. OP-1 induces a dose-dependent increase in
30 osteocalcin production (5-fold increase using 25 ng of OP-1 protein/ml), and a 20-fold increase in osteocalcin mRNA. Agonists are expected to have similar induction effects; antagonists are expected to inhibit or otherwise interfere

with OP1 binding, thereby substantially interfering with osteocalcin induction in the presence of a limiting amount of OP1.

Mineralization is determined on long term cultures (13 day) using a modified von Kossa staining technique on fixed cell layers: cells are fixed
5 in fresh 4% paraformaldehyde at 23°C for 10 min, following rinsing cold 0.9% NaCl. Fixed cells then are stained for endogenous alkaline phosphatase at pH 9.5 for 10 min, using a commercially available kit (Sigma, Inc.) Purple stained cells then are dehydrated with methanol and air dried. After 30 min incubation in 3% AgNO₃ in the dark, H₂O-rinsed samples are exposed for 30 sec
10 to 254 nm UV light to develop the black silver-stained phosphate nodules. Individual mineralized foci (at least 20 nm in size) are counted under a dissecting microscope and expressed as nodules/culture. OP-1 induces a 20-fold increase in initial mineralization rate. Agonists are expected to have similar induction effects; antagonists are expected to inhibit or otherwise
15 interfere with OP1 binding, thereby inhibiting mineralization induction in the presence of a limiting amount of OP1.

9.2 Induction of a Constructed Reporter Gene

Alternatively, a reporter gene construct can be used to determine the ability of candidate molecule to induce signal transduction across a membrane
20 following receptor binding. For example, PAI-1 protein, (Plasminogen Activator Inhibitor-1) expression can be induced by OP-1 in Mv1Lu- cells (see above). Also, as demonstrated above, these cells express ALK-2, -3 and -6 surface receptors. In addition, preliminary studies indicate that ALK-1, when overexpressed in a chemically mutagenized derivative of these cells, also
25 apparently mediates PAI-1 induction in the presence of OP1.

Accordingly, PAI-1 promoter elements can be fused to a reporter gene and induction of the reporter gene monitored following incubation of the transfected cell with a candidate analog. As one example, the luciferase reporter gene can be used, in, for example, the construct p3TP-Lux described
30 by Wrana et al. (1992) Cell 71: 1003-1014 and Attisano et al. (1993) Cell 74: 671-680. This reporter gene construct includes a region of the human PAI-1

- 51 -

gene promoter in combination with three sets of tetradecanoyl phorbol - acetate responsive elements upstream of the luciferase open reading frame.

In a typical assay, transfected cells starved in DMEM containing 0.1% fetal bovine serum and antibiotics (e.g., 100 units/ml penicillin and 50 µg/ml streptomycin) for 6 hrs., and then exposed to ligand for 24 hr. Luciferase activity in the cell lysate then is measured using a luminometer in the luciferase assay system, according to the manufacturer's protocol (Promega). In Mv1Lu mutant cells, "R mutant" cells co-transfected with ALK-2 and Act RII, OP1 mediated induction of luciferase activity.

9.3 Inhibition of Epithelial cell proliferation

OP1 is known to inhibit epithelial cells. Thus, the ability of a candidate analog to inhibit cell proliferation, as measured by ³H-thymidine uptake by an epithelial cell can be used in an assay to evaluate signal transduction activity of the candidate. Analogs competent to inhibit epithelial cell growth are contemplated to have particular utility in therapeutic applications where limitation of a proliferating cell population is desired. Such applications include chemotherapies and radiation therapies where limiting the growth of a normally proliferating population of cells can protect these cells from the cytotoxic effects of these cancer therapies. (see e.g., WO94/06420). In addition, psoriasis and other tissue disorders resulting from uncontrolled cell proliferation, including benign and malignant neoplasias, can be modulated by use of an OP1 analog.

As an example, mink lung epithelial cell growth is inhibited by OP-1. (see, PCT US93/08885; WO94/06420.) As described above, derivatives of these cells [e.g., "R-4 mutants", clone 4-2, Laiho et al. (1990) J. Biol. Chem. 265: 18518-18524] can be transfected with DNA encoding OP1-specific receptors and induced to express these receptors. The transfected cells, then can be assayed for a candidate analog's ability to block cell growth. As one example, when R-4 cells are transfected with ALK-3 under a Zn²⁺-inducible promoter, and induced to express the receptor following induction with ZnCl₂, cell growth can be inhibited in the presence of OP1 in a dose dependent

manner. Preliminary experiments with ALK-1 indicates that this receptor also can mediate this OP-1-specific effect.

In a typical assay, cells are seeded in 24-well cell culture plates at a density of 10^4 cells per well in DMEM with 10 % FBS, and incubated overnight. The medium is replaced with DMEM containing 0.2 % FBS and 100uM ZnLC₂, and the cells are incubated for 5 h, after which the medium is replaced with fresh DMEM containing 0.2 % FBS, 100uM ZnCL₂ and various concentrations of OP-1 or an analog candidate. After 16 h of incubation, 0.25 ci of [³H]thymidine (Amersham) are added and the cells incubated for an additional 2 h. Thereafter, the cells are fixed in 10% trichloroacetic acid for more than 15 min on ice, and solubilized with 1 M NaOH. The cell extracts are neutralized with 1 M HCl and ³H radioactivity determined in a liquid scintillation counter.

Example 10. SCREENING ASSAY FOR COMPOUNDS WHICH ALTER ENDOGENOUS OP1 RECEPTOR EXPRESSION LEVELS

Candidate compound(s) which can be administered to affect the level of a given endogenous OP1 receptor can be found using the following screening assay, in which the level of OP1 receptor production by a cell type which produces measurable levels of the receptor is determined by incubating the cell in culture with and without the candidate compound, in order to assess the effects of the compound on the cell. This also can be accomplished by detection of the OP1 receptor either at the protein level by Western blot or immunolocalization, or at the RNA level by Northern blot or in situ hybridization. The protocol is based on a procedure for identifying compounds which alter endogenous levels of OP1 expression, a detailed description also may be found in PCT US 92/07359, incorporated herein by reference.

Cell cultures of, for example, bone, brain, intestine, lung, heart, eye, breast, gonads, kidney, adrenals, urinary bladder, brain, or other organs, may be prepared as described widely in the literature. For example, kidneys may be explanted from neonatal or new born or young or adult rodents (mouse or rat) and used in organ culture as whole or sliced (1-4 mm) tissues.

Primary tissue cultures and established cell lines, also derived from kidney, adrenals, urinary, bladder, brain, mammary, or other tissues may be established in multiwell plates (6 well or 24 well) according to conventional cell culture techniques, and are cultured in the absence or presence of serum for a period of time (1-7 days). Cells can be cultured, for example, in Dulbecco's Modified Eagle medium (Gibco, Long Island, NY) containing serum (e.g., fetal calf serum at 1%-10%, Gibco) or in serum-deprived medium, as desired, or in defined medium (e.g., containing insulin, transferrin, glucose, albumin, or other growth factors).

Cell samples for testing the level of OP1 receptor production are collected periodically and evaluated for receptor production by immunoblot analysis (Sambrook et al., eds., 1989, Molecular Cloning, Cold Spring Harbor Press, Cold Spring Harbor, NY), or, alternatively, a portion of the cell culture itself can be collected periodically and used to prepare polyA⁺ RNA for mRNA analysis by Northern blot analysis. To monitor *de novo* receptor synthesis, some cultures are labeled according to conventional procedures with an ³⁵S-methionine/³⁵S-cysteine mixture for 6-24 hours and then evaluated to quantitate receptor synthesis by conventional immunoassay methods. Alternatively, anti-receptor antibodies may be labelled and incubated with the cells or cell lysates, and the bound complexes detected and quantitated by conventional means, such as those described hereinabove. Northern blots may be performed using a portion of the OP1 receptor coding sequence to create hybridization probes, and following the RNA hybridization protocol described herein.

Example 11. GENERAL FORMULATION/ ADMINISTRATION CONSIDERATIONS

The analogs and constructs described herein can be provided to an individual as part of a therapy to enhance, inhibit, or otherwise modulate the in vivo binding interaction between OP1 and one or more OP1-specific cell surface receptors. The molecules then comprise part of a pharmaceutical composition as described herein below and can be administered by any suitable means, preferably directly or systemically, e.g., parenterally or orally. Where the therapeutic molecule is to be provided directly (e.g., locally, as

by injection, to a desired tissue site), or parenterally, such as by intravenous, subcutaneous, intramuscular, intraorbital, ophthalmic, intraventricular, intracranial, intracapsular, intraspinal, intracisternal, intraperitoneal, buccal, rectal, vaginal, intranasal or by aerosol administration, the therapeutic preferably comprises part of an aqueous solution. The solution preferably is physiologically acceptable so that in addition to delivery of the desired morphogen to the patient, the solution does not otherwise adversely affect the patient's electrolyte and volume balance. The aqueous medium for the therapeutic molecule thus may comprise normal physiologic saline (0.9% NaCl, 0.15M), pH 7-7.4 or other pharmaceutically acceptable salts thereof.

Useful solutions for oral or parenteral administration can be prepared by any of the methods well known in the pharmaceutical art, described, for example, in Remington's Pharmaceutical Sciences, (Gennaro, A., ed.), Mack Pub., 1990. Formulations may include, for example, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes, and the like. Formulations for direct administration, in particular, can include glycerol and other compositions of high viscosity. Biocompatible, preferably bioresorbable polymers, including, for example, hyaluronic acid, collagen, tricalcium phosphate, polybutyrate, polylactide, polyglycolide and lactide/glycolide copolymers, may be useful excipients to control the release of the morphogen in vivo.

Other potentially useful parenteral delivery systems for these therapeutic molecules include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. Formulations for inhalation administration may contain as excipients, for example, lactose, or can be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or oily solutions for administration in the form of nasal drops, or as a gel to be applied intranasally.

Alternatively, the morphogens described herein may be administered orally.

The therapeutic molecules also can be associated with means for targeting the therapeutic to a desired tissue. For example, tetracycline and diphosphonates (bisphosphonates) are known to bind to bone mineral, particularly at zones of bone remodeling, when they are provided systemically
5 in a mammal. Accordingly, these molecules may be included as useful agents for targeting therapeutics to bone tissue. Alternatively, an antibody or other binding protein that interacts specifically with a surface molecule on the desired target tissue cells also can be used. Such targeting molecules further can be covalently associated to the therapeutic molecule e.g., by
10 chemical crosslinking, or by using standard genetic engineering means to create, for example, an acid labile bond such as an Asp-Pro linkage. Useful targeting molecules can be designed, for example, using the single chain binding site technology disclosed, for example, in U.S. Pat. No. 5,091,513.

Finally, therapeutic molecules can be administered alone or in
15 combination with other molecules known to have a beneficial effect on tissue morphogenesis, including molecules capable of tissue repair and regeneration and/or inhibiting inflammation. Examples of useful cofactors for stimulating bone tissue growth in osteoporotic individuals, for example, include but are not limited to, vitamin D₃, calcitonin, prostaglandins, parathyroid hormone,
20 dexamethasone, estrogen and IGF-I or IGF-II. Useful cofactors for nerve tissue repair and regeneration can include nerve growth factors. Other useful cofactors include symptom-alleviating cofactors, including antiseptics, antibiotics, antiviral and antifungal agents and analgesics and anesthetics.

Therapeutic molecules further can be formulated into pharmaceutical
25 compositions by admixture with pharmaceutically acceptable nontoxic excipients and carriers. As noted above, such compositions can be prepared for parenteral administration, particularly in the form of liquid solutions or suspensions; for oral administration, particularly in the form of tablets or capsules; or intranasally, particularly in the form of powders, nasal drops or
30 aerosols. Where adhesion to a tissue surface is desired the composition may include the morphogen dispersed in a fibrinogen-thrombin composition or other bioadhesive such as is disclosed, for example in PCT US91/09275, the

disclosure of which is incorporated herein by reference. The composition then can be painted, sprayed or otherwise applied to the desired tissue surface.

The compositions can be formulated for parenteral or oral administration to humans or other mammals in therapeutically effective amounts, e.g., amounts which provide appropriate concentrations of the analog to target tissue for a time sufficient to induce the desired effect.

Where the analog is to be used as part of a transplant procedure, it can be provided to the living tissue or organ to be transplanted prior to removal of tissue or organ from the donor. The analog may be provided to the donor host directly, as by injection of a formulation comprising the analog into the tissue, or indirectly, e.g., by oral or parenteral administration, using any of the means described above.

Alternatively or, in addition, once removed from the donor, the organ or living tissue can be placed in a preservation solution containing the therapeutic molecule. In addition, the recipient also preferably is provided with the analog just prior to, or concomitant with, transplantation. In all cases, the analog can be administered directly to the tissue at risk, as by injection to the tissue, or it may be provided systemically, either by oral or parenteral administration, using any of the methods and formulations described herein and/or known in the art.

Where the therapeutic molecule comprises part of a tissue or organ preservation solution, any commercially available preservation solution can be used to advantage. For example, useful solutions known in the art include Collins solution, Wisconsin solution, Belzer solution, Eurocollins solution and lactated Ringer's solution. Generally, an organ preservation solution usually possesses one or more of the following properties: (a) an osmotic pressure substantially equal to that of the inside of a mammalian cell, (solutions typically are hyperosmolar and have K⁺ and/or Mg⁺⁺ ions present in an amount sufficient to produce an osmotic pressure slightly higher than the inside of a mammalian cell); (b) the solution typically is capable of maintaining substantially normal ATP levels in the cells; and (c) the solution usually allows optimum maintenance of glucose metabolism in the cells. Organ

preservation solutions also may contain anticoagulants, energy sources such as glucose, fructose and other sugars, metabolites, heavy metal chelators, glycerol and other materials of high viscosity to enhance survival at low temperatures, free oxygen radical inhibiting and/or scavenging agents and a pH indicator. A detailed description of preservation solutions and useful components can be found, for example, in US Patent No. 5,002,965, the disclosure of which is incorporated herein by reference.

As will be appreciated by those skilled in the art, the concentration of the compounds described in a therapeutic composition will vary depending upon a number of factors, including the dosage of the drug to be administered, the chemical characteristics (e.g., hydrophobicity) of the compounds employed, and the route of administration. The preferred dosage of drug to be administered also is likely to depend on such variables as the type and extent of tissue loss or defect, the overall health status of the particular patient, the relative biological efficacy of the compound selected, the formulation of the compound, the presence and types of excipients in the formulation, and the route of administration. In general terms, the therapeutic molecules of this invention may be provided to an individual where typical dose ranges are from about 10 ng/kg to about 1 g/kg of body weight per day; a preferred dose range being from about 0.1 mg/kg to 100 mg/kg of body weight. No obvious morphogen-induced pathological lesions are induced when mature morphogen (e.g., OP-1, 20 mg) is administered daily to normal growing rats for 21 consecutive days. Moreover, 10 mg systemic injections of morphogen (e.g., OP-1) injected daily for 10 days into normal newborn mice does not produce any gross abnormalities.

25 Other Embodiments

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- 5 (i) APPLICANT:
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10 (E) COUNTRY: USA
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(H) TELEFAX:
(I) TELEX:
- 25 (ii) TITLE OF INVENTION: MORPHOGENIC PROTEIN-SPECIFIC CELL
SURFACE RECEPTORS AND USES THEREFOR
- 30 (iii) NUMBER OF SEQUENCES: 15
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: PATENT ADMINISTRATOR; TESTA, HURWITZ &
THIBEAULT
(B) STREET: 53 STATE STREET
35 (C) CITY: BOSTON
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02109
- 40 (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- 45 (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
- 50 (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: PITCHER, EDMUND R.
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- 60

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1509 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 1..1509
 (D) OTHER INFORMATION: /product= "Human ALK1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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	1 5 10 15	
25	TTG GTG ACC CAG GGA GAC CCT GTG AAG CCG TCT CGG GGC CCG CTG GTG	96
	Leu Val Thr Gln Gly Asp Pro Val Lys Pro Ser Arg Gly Pro Leu Val	
	20 25 30	
30	ACC TGC ACG TGT GAG AGC CCA CAT TGC AAG GGG CCT ACC TGC CGG GGG	144
	Thr Cys Thr Cys Glu Ser Pro His Cys Lys Gly Pro Thr Cys Arg Gly	
	35 40 45	
35	GCC TGG TGC ACA GTA GTG CTG GTG CGG GAG GAG GGG AGG CAC CCC CAG	192
	Ala Trp Cys Thr Val Val Leu Val Arg Glu Glu Gly Arg His Pro Gln	
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40	GAA CAT CGG GGC TGC GGG AAC TTG CAC AGG GAG CTC TGC AGG GGG CGC	240
	Glu His Arg Gly Cys Gly Asn Leu His Arg Glu Leu Cys Arg Gly Arg	
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45	CCC ACC GAG TTC GTC AAC CAC TAC TGC TGC GAC AGC CAC CTC TGC AAC	288
	Pro Thr Glu Phe Val Asn His Tyr Cys Cys Asp Ser His Leu Cys Asn	
	85 90 95	
50	CAC AAC GTG TCC CTG GTG CTG GAG GCC ACC CAA CCT CCT TCG GAG CAG	336
	His Asn Val Ser Leu Val Leu Glu Ala Thr Gln Pro Pro Ser Glu Gln	
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55	CCG GGA ACA GAT GGC CAG CTG GCC CTG ATC CTG GGC CCC GTG CTG GCC	384
	Pro Gly Thr Asp Gly Gln Leu Ala Leu Ile Leu Gly Pro Val Leu Ala	
	115 120 125	
60	TTG CTG GCC CTG GTG GCC CTG GGT GTC CTG GGC CTG TGG CAT GTC CGA	432
	Leu Leu Ala Leu Val Ala Leu Gly Val Leu Gly Leu Trp His Val Arg	
	130 135 140	
65	CGG AGG CAG GAG AAG CAG CGT GGC CTG CAC AGC GAG CTG GGA GAG TCC	480
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	Ser	Leu	Ile	Leu	Lys	Ala	Ser	Glu	Gln	Gly	Asp	Thr	Met	Leu	Gly	Asp	
					165					170					175		
5	CTC	CTG	GAC	AGT	GAC	TGC	ACC	ACA	GGG	AGT	GGC	TCA	GGG	CTC	CCC	TTC	576
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15	GGA	AAA	GGC	CGC	TAT	GGC	GAA	GTG	TGG	CGG	GGC	TTG	TGG	CAC	GGT	GAG	672
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		225				230					235					240	
25	CGG	GAG	ACT	GAG	ATC	TAT	AAC	ACA	GTA	TTG	CTC	AGA	CAC	GAC	AAC	ATC	768
	Arg	Glu	Thr	Glu	Ile	Tyr	Asn	Thr	Val	Leu	Leu	Arg	His	Asp	Asn	Ile	
					245					250					255		
30	CTA	GGC	TTC	ATC	GCC	TCA	GAC	ATG	ACC	TCC	CGC	AAC	TCG	AGC	ACG	CAG	816
	Leu	Gly	Phe	Ile	Ala	Ser	Asp	Met	Thr	Ser	Arg	Asn	Ser	Ser	Thr	Gln	
				260					265					270			
35	CTG	TGG	CTC	ATC	ACG	CAC	TAC	CAC	GAG	CAC	GGC	TCC	CTC	TAC	GAC	TTT	864
	Leu	Trp	Leu	Ile	Thr	His	Tyr	His	Glu	His	Gly	Ser	Leu	Tyr	Asp	Phe	
			275					280					285				
40	CTG	CAG	AGA	CAG	ACG	CTG	GAG	CCC	CAT	CTG	GCT	CTG	AGG	CTA	GCT	GTG	912
	Leu	Gln	Arg	Gln	Thr	Leu	Glu	Pro	His	Leu	Ala	Leu	Arg	Leu	Ala	Val	
		290					295					300					
45	TCC	CGC	GCA	TGC	GGC	CTG	GCG	CAC	CTG	CAC	GTG	GAG	ATC	TTC	GGT	ACA	960
	Ser	Ala	Ala	Cys	Gly	Leu	Ala	His	Leu	His	Val	Glu	Ile	Phe	Gly	Thr	
		305				310					315					320	
50	CAG	GGC	AAA	CCA	GCC	ATT	GCC	CAC	CGC	GAC	TTC	AAG	AGC	CGC	AAT	GTG	1008
	Gln	Gly	Lys	Pro	Ala	Ile	Ala	His	Arg	Asp	Phe	Lys	Ser	Arg	Asn	Val	
					325					330					335		
55	CTG	GTC	AAG	AGC	AAC	CTG	CAG	TGT	TGC	ATC	GCC	GAC	CTG	GGC	CTG	GCT	1056
	Leu	Val	Lys	Ser	Asn	Leu	Gln	Cys	Cys	Ile	Ala	Asp	Leu	Gly	Leu	Ala	
				340				345						350			
60	GTG	ATG	CAC	TCA	CAG	GGC	AGC	GAT	TAC	CTG	GAC	ATC	GGC	AAC	AAC	CCG	1104
	Val	Met	His	Ser	Gln	Gly	Ser	Asp	Tyr	Leu	Asp	Ile	Gly	Asn	Asn	Pro	
			355					360					365				
65	AGA	GTG	GGC	ACC	AAG	CGG	TAC	ATG	GCA	CCC	GAG	GTG	CTG	GAC	GAG	CAG	1152
	Arg	Val	Gly	Thr	Lys	Arg	Tyr	Met	Ala	Pro	Glu	Val	Leu	Asp	Glu	Gln	
		370					375					380					
70	ATC	CGC	ACG	GAC	TGC	TTT	GAG	TCC	TAC	AAG	TGG	ACT	GAC	ATC	TGG	GCC	1200
	Ile	Arg	Thr	Asp	Cys	Phe	Glu	Ser	Tyr	Lys	Trp	Thr	Asp	Ile	Trp	Ala	
		385				390					395					400	
75	TTT	GGC	CTG	GTG	CTG	TGG	GAG	ATT	GCC	CGC	CGG	ACC	ATC	GTG	AAT	GGC	1248

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Phe Gly Leu Val Leu Trp Glu Ile Ala Arg Arg Thr Ile Val Asn Gly
 405 410 415

5 ATC GTG GAG GAC TAT AGA CCA CCC TTC TAT GAT GTG GTG CCC AAT GAC 1296
 Ile Val Glu Asp Tyr Arg Pro Pro Phe Tyr Asp Val Val Pro Asn Asp
 420 425 430

10 CCC AGC TTT GAG GAC ATG AAG AAG GTG GTG TGT GTG GAT CAG CAG ACC 1344
 Pro Ser Phe Glu Asp Met Lys Lys Val Val Cys Val Asp Gln Gln Thr
 435 440 445

15 CCC ACC ATC CCT AAC CGG CTG GCT GCA GAC CCG GTC CTC TCA GGC CTA 1392
 Pro Thr Ile Pro Asn Arg Leu Ala Ala Asp Pro Val Leu Ser Gly Leu
 450 455 460

20 GCT CAG ATG ATG CGG GAG TGC TGG TAC CCA AAC CCC TCT GCC CGA CTC 1440
 Ala Gln Met Met Arg Glu Cys Trp Tyr Pro Asn Pro Ser Ala Arg Leu
 465 470 475 480

25 ACC GCG CTG CGG ATC AAG AAG ACA CTA CAA AAA ATT AGC AAC AGT CCA 1488
 Thr Ala Leu Arg Ile Lys Lys Thr Leu Gln Lys Ile Ser Asn Ser Pro
 485 490 495

30 GAG AAG CCT AAA GTG ATT CAA 1509
 Glu Lys Pro Lys Val Ile Gln
 500

(2) INFORMATION FOR SEQ ID NO:2:

30

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 503 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

40 Met Thr Leu Gly Ser Pro Arg Lys Gly Leu Leu Met Leu Leu Met Ala
 1 5 10 15

Leu Val Thr Gln Gly Asp Pro Val Lys Pro Ser Arg Gly Pro Leu Val
 20 25 30

45 Thr Cys Thr Cys Glu Ser Pro His Cys Lys Gly Pro Thr Cys Arg Gly
 35 40 45

50 Ala Trp Cys Thr Val Val Leu Val Arg Glu Glu Gly Arg His Pro Gln
 50 55 60

Glu His Arg Gly Cys Gly Asn Leu His Arg Glu Leu Cys Arg Gly Arg
 65 70 75 80

55 Pro Thr Glu Phe Val Asn His Tyr Cys Cys Asp Ser His Leu Cys Asn
 85 90 95

His Asn Val Ser Leu Val Leu Glu Ala Thr Gln Pro Pro Ser Glu Gln
 100 105 110

60

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Pro Gly Thr Asp Gly Gln Leu Ala Leu Ile Leu Gly Pro Val Leu Ala
 115 120 125
 5 Leu Leu Ala Leu Val Ala Leu Gly Val Leu Gly Leu Trp His Val Arg
 130 135 140
 Arg Arg Gln Glu Lys Gln Arg Gly Leu His Ser Glu Leu Gly Glu Ser
 145 150 155 160
 10 Ser Leu Ile Leu Lys Ala Ser Glu Gln Gly Asp Thr Met Leu Gly Asp
 165 170 175
 Leu Leu Asp Ser Asp Cys Thr Thr Gly Ser Gly Ser Gly Leu Pro Phe
 180 185 190
 15 Leu Val Gln Arg Thr Val Ala Arg Gln Val Ala Leu Val Glu Cys Val
 195 200 205
 Gly Lys Gly Arg Tyr Gly Glu Val Trp Arg Gly Leu Trp His Gly Glu
 210 215 220
 20 Ser Val Ala Val Lys Ile Phe Ser Ser Arg Asp Glu Gln Ser Trp Phe
 225 230 235 240
 25 Arg Glu Thr Glu Ile Tyr Asn Thr Val Leu Leu Arg His Asp Asn Ile
 245 250 255
 Leu Gly Phe Ile Ala Ser Asp Met Thr Ser Arg Asn Ser Ser Thr Gln
 260 265 270
 30 Leu Trp Leu Ile Thr His Tyr His Glu His Gly Ser Leu Tyr Asp Phe
 275 280 285
 Leu Gln Arg Gln Thr Leu Glu Pro His Leu Ala Leu Arg Leu Ala Val
 290 295 300
 35 Ser Ala Ala Cys Gly Leu Ala His Leu His Val Glu Ile Phe Gly Thr
 305 310 315 320
 40 Gln Gly Lys Pro Ala Ile Ala His Arg Asp Phe Lys Ser Arg Asn Val
 325 330 335
 Leu Val Lys Ser Asn Leu Gln Cys Cys Ile Ala Asp Leu Gly Leu Ala
 340 345 350
 45 Val Met His Ser Gln Gly Ser Asp Tyr Leu Asp Ile Gly Asn Asn Pro
 355 360 365
 Arg Val Gly Thr Lys Arg Tyr Met Ala Pro Glu Val Leu Asp Glu Gln
 370 375 380
 50 Ile Arg Thr Asp Cys Phe Glu Ser Tyr Lys Trp Thr Asp Ile Trp Ala
 385 390 395 400
 55 Phe Gly Leu Val Leu Trp Glu Ile Ala Arg Arg Thr Ile Val Asn Gly
 405 410 415
 Ile Val Glu Asp Tyr Arg Pro Pro Phe Tyr Asp Val Val Pro Asn Asp
 420 425 430
 60

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Pro Ser Phe Glu Asp Met Lys Lys Val Val Cys Val Asp Gln Gln Thr
 435 440 445

5 Pro Thr Ile Pro Asn Arg Leu Ala Ala Asp Pro Val Leu Ser Gly Leu
 450 455 460

Ala Gln Met Met Arg Glu Cys Trp Tyr Pro Asn Pro Ser Ala Arg Leu
 465 470 475 480

10 Thr Ala Leu Arg Ile Lys Lys Thr Leu Gln Lys Ile Ser Asn Ser Pro
 485 490 495

Glu Lys Pro Lys Val Ile Gln
 500

15

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2724 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 104..1633
 (D) OTHER INFORMATION: /product= "Human ALK2"

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTCCGAGTAC CCCAGTGACC AGAGTGAGAG AAGCTCTGAA CGAGGGCACG CGGCTTGAAG 60

GACTGTGGGC AGATGTGACC AAGAGCCTGC ATTAAGTTGT ACA ATG GTA GAT GGA 115
 Met Val Asp Gly
 1

40 GTG ATG ATT CTT CCT GTG CTT ATC ATG ATT GCT CTC CCC TCC CCT AGT 163
 Val Met Ile Leu Pro Val Leu Ile Met Ile Ala Leu Pro Ser Pro Ser
 5 10 15 20

45 ATG GAA GAT GAG AAG CCC AAG GTC AAC CCC AAA CTC TAC ATG TGT GTG 211
 Met Glu Asp Glu Lys Pro Lys Val Asn Pro Lys Leu Tyr Met Cys Val
 25 30 35

50 TGT GAA GGT CTC TCC TGC GGT AAT GAG GAC CAC TGT GAA GGC CAG CAG 259
 Cys Glu Gly Leu Ser Cys Gly Asn Glu Asp His Cys Glu Gly Gln Gln
 40 45 50

55 TGC TTT TCC TCA CTG AGC ATC AAC GAT GGC TTC CAC GTC TAC CAG AAA 307
 Cys Phe Ser Ser Leu Ser Ile Asn Asp Gly Phe His Val Tyr Gln Lys
 55 60 65

GGC TGC TTC CAG GTT TAT GAG CAG GGA AAG ATG ACC TGT AAG ACC CCG 355
 Gly Cys Phe Gln Val Tyr Glu Gln Gly Lys Met Thr Cys Lys Thr Pro
 60 70 75 80

	CCG	TCC	CCT	GGC	CAA	GCT	GTG	GAG	TGC	TGC	CAA	GGG	GAC	TGG	TGT	AAC	403
	Pro	Ser	Pro	Gly	Gln	Ala	Val	Glu	Cys	Cys	Gln	Gly	Asp	Trp	Cys	Asn	
	85					90					95					100	
5	AGG	AAC	ATC	ACG	GCC	CAG	CTG	CCC	ACT	AAA	GGA	AAA	TCC	TTC	CCT	GGA	451
	Arg	Asn	Ile	Thr	Ala	Gln	Leu	Pro	Thr	Lys	Gly	Lys	Ser	Phe	Pro	Gly	
					105					110					115		
10	ACA	CAG	AAT	TTC	CAC	TTG	GAG	GTT	GGC	CTC	ATT	ATT	CTC	TCT	GTA	GTG	499
	Thr	Gln	Asn		His	Leu	Glu	Val	Gly	Leu	Ile	Ile	Leu	Ser	Val	Val	
				120					125					130			
15	TTC	GCA	GTA	TGT	CTT	TTA	GCC	TGC	CTG	CTG	GGA	GTT	GCT	CTC	CGA	AAA	547
	Phe	Ala	Val	Cys	Leu	Leu	Ala	Cys	Leu	Leu	Gly	Val	Ala	Leu	Arg	Lys	
			135					140					145				
20	TTT	AAA	AGG	CGC	AAC	CAA	GAA	CGC	CTC	AAT	CCC	CGA	GAC	GTG	GAG	TAT	595
	Phe	Lys	Arg	Arg	Asn	Gln	Glu	Arg	Leu	Asn	Pro	Arg	Asp	Val	Glu	Tyr	
	150					155						160					
25	GGC	ACT	ATC	GAA	GGG	CTC	ATC	ACC	ACC	AAT	GTT	GGA	GAC	AGC	ACT	TTA	643
	Gly	Thr	Ile	Glu	Gly	Leu	Ile	Thr	Thr	Asn	Val	Gly	Asp	Ser	Thr	Leu	
	165					170					175					180	
30	GCA	GAT	TTA	TTG	GAT	CAT	TCG	TGT	ACA	TCA	GGA	AGT	GGC	TCT	GGT	CTT	691
	Ala	Asp	Leu	Leu	Asp	His	Ser	Cys	Thr	Ser	Gly	Ser	Gly	Ser	Gly	Leu	
					185					190					195		
35	CCT	TTT	CTG	GTA	CAA	AGA	ACA	GTG	GCT	CGC	CAG	ATT	ACA	CTG	TTG	GAG	739
	Pro	Phe	Leu	Val	Gln	Arg	Thr	Val	Ala	Arg	Gln	Ile	Thr	Leu	Leu	Glu	
				200				205						210			
40	TGT	GTC	GGG	AAA	GGC	AGG	TAT	GGT	GAG	GTG	TGG	AGG	GGC	AGC	TGG	CAA	787
	Cys	Val	Gly	Lys	Gly	Arg	Tyr	Gly	Glu	Val	Trp	Arg	Gly	Ser	Trp	Gln	
			215					220					225				
45	GGG	GAA	AAT	GTT	GCC	GTG	AAG	ATC	TTC	TCC	TCC	CGT	GAT	GAG	AAG	TCA	835
	Gly	Glu	Asn	Val	Ala	Val	Lys	Ile	Phe	Ser	Ser	Arg	Asp	Glu	Lys	Ser	
	230					235						240					
50	TGG	TTC	AGG	GAA	ACG	GAA	TTG	TAC	AAC	ACT	GTG	ATG	CTG	AGG	CAT	GAA	883
	Trp	Phe	Arg	Glu	Thr	Glu	Leu	Tyr	Asn	Thr	Val	Met	Leu	Arg	His	Glu	
	245					250					255					260	
55	AAT	ATC	TTA	GGT	TTC	ATT	GCT	TCA	GAC	ATG	ACA	TCA	AGA	CAC	TCC	AGT	931
	Asn	Ile	Leu	Gly	Phe	Ile	Ala	Ser	Asp	Met	Thr	Ser	Arg	His	Ser	Ser	
					265					270					275		
60	ACC	CAG	CTG	TGG	TTA	ATT	ACA	CAT	TAT	CAT	GAA	ATG	GGA	TCG	TTG	TAC	979
	Thr	Gln	Leu	Trp	Leu	Ile	Thr	His	Tyr	His	Glu	Met	Gly	Ser	Leu	Tyr	
				280				285						290			
65	GAC	TAT	CTT	CAG	CTT	ACT	ACT	CTG	GAT	ACA	GTT	AGC	TGC	CTT	CGA	ATA	1027
	Asp	Tyr	Leu	Gln	Leu	Thr	Thr	Leu	Asp	Thr	Val	Ser	Cys	Leu	Arg	Ile	
			295					300					305				
70	GTG	CTG	TCC	ATA	GCT	AGT	GGT	CTT	GCA	CAT	TTG	CAC	ATA	GAG	ATA	TTT	1075
	Val	Leu	Ser	Ile	Ala	Ser	Gly	Leu	Ala	His	Leu	His	Ile	Glu	Ile	Phe	
			310				315					320					

	GGG ACC CAA GGG AAA CCA GCC ATT GCC CAT CGA GAT TTA AAG AGC AAA	1123
	Gly Thr Gln Gly Lys Pro Ala Ile Ala His Arg Asp Leu Lys Ser Lys	
	325 330 335 340	
5	AAT ATT CTG GTT AAG AAG AAT GGA CAG TGT TGC ATA GCA GAT TTG GGC	1171
	Asn Ile Leu Val Lys Lys Asn Gly Gln Cys Ile Ala Asp Leu Gly	
	345 350 355	
10	CTG GCA GTC ATG CAT TCC CAG AGC ACC AAT CAG CTT GAT GTG GGG AAC	1219
	Leu Ala Val Met His Ser Gln Ser Thr Asn Gln Leu Asp Val Gly Asn	
	360 365 370	
15	AAT CCC CGT GTG GGC ACC AAG CGC TAC ATG GCC CCC GAA GTT CTA GAT	1267
	Asn Pro Arg Val Gly Thr Lys Arg Tyr Met Ala Pro Glu Val Leu Asp	
	375 380 385	
20	GAA ACC ATC CAG GTG GAT TGT TTC GAT TCT TAT AAA AGG GTC GAT ATT	1315
	Glu Thr Ile Gln Val Asp Cys Phe Asp Ser Tyr Lys Arg Val Asp Ile	
	390 395 400	
25	TGG GCC TTT GGA CTT GTT TTG TGG GAA GTG GCC AGG CGG ATG GTG AGC	1363
	Trp Ala Phe Gly Leu Val Leu Trp Glu Val Ala Arg Arg Met Val Ser	
	405 410 415 420	
30	AAT GGT ATA GTG GAG GAT TAC AAG CCA CCG TTC TAC GAT GTG GTT CCC	1411
	Asn Gly Ile Val Glu Asp Tyr Lys Pro Phe Tyr Asp Val Val Pro	
	425 430 435	
35	AAT GAC CCA AGT TTT GAA GAT ATG AGG AAG GTA GTC TGT GTG GAT CAA	1459
	Asn Asp Pro Ser Phe Glu Asp Met Arg Lys Val Val Cys Val Asp Gln	
	440 445 450	
40	CAA AGG CCA AAC ATA CCC AAC AGA TGG TTC TCA GAC CCG ACA TTA ACC	1507
	Gln Arg Pro Asn Ile Pro Asn Arg Trp Phe Ser Asp Pro Thr Leu Thr	
	455 460 465	
45	TCT CTG GCC AAG CTA ATG AAA GAA TGC TGG TAT CAA AAT CCA TCC GCA	1555
	Ser Leu Ala Lys Leu Met Lys Glu Cys Trp Tyr Gln Asn Pro Ser Ala	
	470 475 480	
50	AGA CTC ACA GCA CTG CGT ATC AAA AAG ACT TTG ACC AAA ATT GAT AAT	1603
	Arg Leu Thr Ala Leu Arg Ile Lys Lys Thr Leu Thr Lys Ile Asp Asn	
	485 490 495 500	
55	TCC CTC GAC AAA TTG AAA ACT GAC TGT TGACATTTTC ATAGTGTC	1650
	Ser Leu Asp Lys Leu Lys Thr Asp Cys	
	505 510	
60	GAAGGAAGAT TTGACGTTGT TGTCATTGTC CAGCTGGGAC CTAATGCTGG CCTGACTGGT	1710
	TGTCAGAATG GAATCCATCT GTCTCCCTCC CCAAATGGCT GCTTTGACAA GGCAGACGTC	1770
	GTACCCAGCC ATGTGTTGGG GAGACATCAA AACCACCCTA ACCTCGCTCG ATGACTGTGA	1830
	ACTGGGCATT TCACGAACTG TTCACACTGC AGAGACTAAT GTTGACAGA CACTGTTGCA	1890
	AAGGTAGGGA CTGGAGGAAC ACAGAGAAAT CCTAAAAGAG ATCTGGGCAT TAAGTCAGTG	1950
	GCTTTGCATA GCTTTCACAA GTCTCCTAGA CACTCCCCAC GGGAACTCA AGGAGGTGGT	2010

GAATTTTAA TCAGCAATAT TGCCTGTGCT TCTCTTCTTT ATTGCACTAG GAATTCCTTG 2070
 CATTCCTTAC TTGCACTGTT ACTCTTAATT TTAAAGACCC AACTTGCCAA AATGTTGGCT 2130
 5 GCGTACTCCA CTGGTCTGTC TTTGGATAAT AGGAATTCAA TTTGGCAAAA CAAAATGTAA 2190
 TGTCAGACTT TGCTGCATTT TACACATGTG CTGATGTTTA CAATGATGCC GAACATTAGG 2250
 10 AATTGTTTAT ACACAACCTT GCAAATTATT TATTACTTGT GCACTTAGTA GTTTTTACAA 2310
 AACTGCTTTG TGCATATGTT AAAGCTTATT TTTATGTGGT CTTATGATTT TATTACAGAA 2370
 ATGTTTTTAA CACTATACTC TAAAATGGAC ATTTTCTTTT ATTATCAGTT AAAATCACAT 2430
 15 TTTAAGTGCT TCACATTTGT ATGTGTGTAG ACTGTAACCT TTTTTCAGTT CATATGCAGA 2490
 ACGTATTTAG CCATTACCCA CGTGACACCA CCGAATATAT TATCGATTTA GAAGCAAAGA 2550
 20 TTTCAGTAGA ATTTTAGTCC TGAACGCTAC GGGGAAAATG CATTTTCTTC AGAATTATCC 2610
 ATTACGTGCA TTAAACTCT GCCAGAAAAA AATAACTATT TTGTTTAAAT CTACTTTTGT 2670
 TATTTAGTAG TTATTTGTAT AAATTAAATA AACTGTTTTT AAGTCAAAAA AAAA 2724
 25

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
 30 (A) LENGTH: 509 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
 35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Val Asp Gly Val Met Ile Leu Pro Val Leu Ile Met Ile Ala Leu
 1 5 10 15
 40 Pro Ser Pro Ser Met Glu Asp Glu Lys Pro Lys Val Asn Pro Lys Leu
 20 25 30
 45 Tyr Met Cys Val Cys Glu Gly Leu Ser Cys Gly Asn Glu Asp His Cys
 35 40 45
 Glu Gly Gln Gln Cys Phe Ser Ser Leu Ser Ile Asn Asp Gly Phe His
 50 55 60
 50 Val Tyr Gln Lys Gly Cys Phe Gln Val Tyr Glu Gln Gly Lys Met Thr
 65 70 75 80
 Cys Lys Thr Pro Pro Ser Pro Gly Gln Ala Val Glu Cys Cys Gln Gly
 85 90 95
 55 Asp Trp Cys Asn Arg Asn Ile Thr Ala Gln Leu Pro Thr Lys Gly Lys
 100 105 110
 60 Ser Phe Pro Gly Thr Gln Asn Phe His Leu Glu Val Gly Leu Ile Ile
 115 120 125

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	Leu	Ser	Val	Val	Phe	Ala	Val	Cys	Leu	Leu	Ala	Cys	Leu	Leu	Gly	Val
	130						135					140				
5	Ala	Leu	Arg	Lys	Phe	Lys	Arg	Arg	Asn	Gln	Glu	Arg	Leu	Asn	Pro	Arg
	145					150					155				160	
	Asp	Val	Glu	Tyr	Gly	Thr	Ile	Glu	Gly	Leu	Ile	Thr	Thr	Asn	Val	Gly
					165					170					175	
10	Asp	Ser	Thr	Leu	Ala	Asp	Leu	Leu	Asp	His	Ser	Cys	Thr	Ser	Gly	Ser
				180					185					190		
	Gly	Ser	Gly	Leu	Pro	Phe	Leu	Val	Gln	Arg	Thr	Val	Ala	Arg	Gln	Ile
15			195					200					205			
	Thr	Leu	Leu	Glu	Cys	Val	Gly	Lys	Gly	Arg	Tyr	Gly	Glu	Val	Trp	Arg
	210						215					220				
20	Gly	Ser	Trp	Gln	Gly	Glu	Asn	Val	Ala	Val	Lys	Ile	Phe	Ser	Ser	Arg
	225					230					235					240
	Asp	Glu	Lys	Ser	Trp	Phe	Arg	Glu	Thr	Glu	Leu	Tyr	Asn	Thr	Val	Met
					245					250						255
25	Leu	Arg	His	Glu	Asn	Ile	Leu	Gly	Phe	Ile	Ala	Ser	Asp	Met	Thr	Ser
				260					265					270		
	Arg	His	Ser	Ser	Thr	Gln	Leu	Trp	Leu	Ile	Thr	His	Tyr	His	Glu	Met
30			275					280					285			
	Gly	Ser	Leu	Tyr	Asp	Tyr	Leu	Gln	Leu	Thr	Thr	Leu	Asp	Thr	Val	Ser
		290					295					300				
35	Cys	Leu	Arg	Ile	Val	Leu	Ser	Ile	Ala	Ser	Gly	Leu	Ala	His	Leu	His
	305					310					315					320
	Ile	Glu	Ile	Phe	Gly	Thr	Gln	Gly	Lys	Pro	Ala	Ile	Ala	His	Arg	Asp
					325					330					335	
40	Leu	Lys	Ser	Lys	Asn	Ile	Leu	Val	Lys	Lys	Asn	Gly	Gln	Cys	Cys	Ile
				340					345					350		
	Ala	Asp	Leu	Gly	Leu	Ala	Val	Met	His	Ser	Gln	Ser	Thr	Asn	Gln	Leu
45			355					360						365		
	Asp	Val	Gly	Asn	Asn	Pro	Arg	Val	Gly	Thr	Lys	Arg	Tyr	Met	Ala	Pro
		370					375					380				
50	Glu	Val	Leu	Asp	Glu	Thr	Ile	Gln	Val	Asp	Cys	Phe	Asp	Ser	Tyr	Lys
	385					390					395					400
	Arg	Val	Asp	Ile	Trp	Ala	Phe	Gly	Leu	Val	Leu	Trp	Glu	Val	Ala	Arg
					405					410					415	
55	Arg	Met	Val	Ser	Asn	Gly	Ile	Val	Glu	Asp	Tyr	Lys	Pro	Pro	Phe	Tyr
				420					425					430		
	Asp	Val	Val	Pro	Asn	Asp	Pro	Ser	Phe	Glu	Asp	Met	Arg	Lys	Val	Val
60			435					440					445			

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Cys Val Asp Gln Gln Arg Pro Asn Ile Pro Asn Arg Trp Phe Ser Asp
 450 455 460
 5 Pro Thr Leu Thr Ser Leu Ala Lys Leu Met Lys Glu Cys Trp Tyr Gln
 465 470 475 480
 Asn Pro Ser Ala Arg Leu Thr Ala Leu Arg Ile Lys Lys Thr Leu Thr
 485 490 495
 10 Lys Ile Asp Asn Ser Leu Asp Lys Leu Lys Thr Asp Cys
 500 505
 15 (2) INFORMATION FOR SEQ ID NO:5:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2932 base pairs
 (B) TYPE: nucleic acid
 20 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: cDNA
 25 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 310..1908
 (D) OTHER INFORMATION: /product= "Human ALK3"
 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
 GCTCCGCGCC GAGGGCTGGA GGATGCGTTC CCTGGGGTCC GGACTTATGA AAATATGCAT 60
 35 CAGTTTAATA CTGTCTTGGA ATTCATGAGA TGAAGCATA GGTCAAAGCT GTTTGGAGAA 120
 AATCAGAAGT ACAGTTTAT CTAGCCACAT CTTGGAGGAG TCGTAAGAAA GCAGTGGGAG 180
 40 TTGAAGTCAT TGTCAAGTGC TTGCGATCTT TTACAAGAAA ATCTCACTGA ATGATAGTCA 240
 TTAAATTGG TGAAGTAGCA AGACCAATTA TTAAGGTGA CAGTACACAG GAAACATTAC 300
 AATTGAACA ATG ACT CAG CTA TAC ATT TAC ATC AGA TTA TTG GGA GCC 348
 45 Met Thr Gln Leu Tyr Ile Tyr Ile Arg Leu Leu Gly Ala
 1 5 10
 TAT TTG TTC ATC ATT TCT CGT GTT CAA GGA CAG AAT CTG GAT AGT ATG 396
 50 Tyr Leu Phe Ile Ile Ser Arg Val Gln Gly Gln Asn Leu Asp Ser Met
 15 20 25
 CTT CAT GGC ACT GGG ATG AAA TCA GAC TCC GAC CAG AAA AAG TCA GAA 444
 Leu His Gly Thr Gly Met Lys Ser Asp Ser Asp Gln Lys Lys Ser Glu
 30 35 40 45
 55 AAT GGA GTA ACC TTA GCA CCA GAG GAT ACC TTG CCT TTT TTA AAG TGC 492
 Asn Gly Val Thr Leu Ala Pro Glu Asp Thr Leu Pro Phe Leu Lys Cys
 50 55 60
 60 TAT TGC TCA GGG CAC TGT CCA GAT GAT GCT ATT AAT AAC ACA TGC ATA 540

	Tyr	Cys	Ser	Gly	His	Cys	Pro	Asp	Asp	Ala	Ile	Asn	Asn	Thr	Cys	Ile	
				65					70					75			
5	ACT	AAT	GGA	CAT	TGC	TTT	GCC	ATC	ATA	GAA	GAA	GAT	GAC	CAG	GGA	GAA	588
	Thr	Asn	Gly	His	Cys	Phe	Ala	Ile	Ile	Glu	Glu	Asp	Asp	Gln	Gly	Glu	
			80					85					90				
10	ACC	ACA	TTA	GCT	TCA	GGG	TGT	ATG	AAA	TAT	GAA	GGA	TCT	GAT	TTT	CAG	636
	Thr	Thr	Leu	Ala	Ser	Gly	Cys	Met	Lys	Tyr	Glu	Gly	Ser	Asp	Phe	Gln	
			95				100					105					
15	TGC	AAA	GAT	TCT	CCA	AAA	GCC	CAG	CTA	CGC	CGG	ACA	ATA	GAA	TGT	TGT	684
	Cys	Lys	Asp	Ser	Pro	Lys	Ala	Gln	Leu	Arg	Arg	Thr	Ile	Glu	Cys	Cys	
	110					115					120				125		
20	CGG	ACC	AAT	TTA	TGT	AAC	CAG	TAT	TTG	CAA	CCC	ACA	CTG	CCC	CCT	GTT	732
	Arg	Thr	Asn	Leu	Cys	Asn	Gln	Tyr	Leu	Gln	Pro	Thr	Leu	Pro	Pro	Val	
					130					135					140		
25	GTC	ATA	GGT	CCG	TTT	TTT	GAT	GGC	AGC	ATT	CGA	TGG	CTG	GTT	TTG	CTC	780
	Val	Ile	Gly	Pro	Phe	Phe	Asp	Gly	Ser	Ile	Arg	Trp	Leu	Val	Leu	Leu	
				145				150						155			
30	ATT	TCT	ATG	GCT	GTC	TGC	ATA	ATT	GCT	ATG	ATC	ATC	TTC	TCC	AGC	TGC	828
	Ile	Ser	Met	Ala	Val	Cys	Ile	Ile	Ala	Met	Ile	Ile	Phe	Ser	Ser	Cys	
			160				165						170				
35	TTT	TGT	TAC	AAA	CAT	TAT	TGC	AAG	AGC	ATC	TCA	AGC	AGA	CGT	CGT	TAC	876
	Phe	Cys	Tyr	Lys	His	Tyr	Cys	Lys	Ser	Ile	Ser	Ser	Arg	Arg	Arg	Tyr	
			175				180					185					
40	AAT	CGT	GAT	TTG	GAA	CAG	GAT	GAA	GCA	TTT	ATT	CCA	GTT	GGA	GAA	TCA	924
	Asn	Arg	Asp	Leu	Glu	Gln	Asp	Glu	Ala	Phe	Ile	Pro	Val	Gly	Glu	Ser	
	190					195					200				205		
45	CTA	AAA	GAC	CTT	ATT	GAC	CAG	TCA	CAA	AGT	TCT	GGT	AGT	GGG	TCT	GGA	972
	Leu	Lys	Asp	Leu	Ile	Asp	Gln	Ser	Gln	Ser	Ser	Gly	Ser	Gly	Ser	Gly	
					210					215					220		
50	CTA	CCT	TTA	TTG	GTT	CAG	CGA	ACT	ATT	GCC	AAA	CAG	ATT	CAG	ATG	GTC	1020
	Leu	Pro	Leu	Leu	Val	Gln	Arg	Thr	Ile	Ala	Lys	Gln	Ile	Gln	Met	Val	
					225				230					235			
55	CGG	CAA	GTT	GGT	AAA	GGC	CGA	TAT	GGA	GAA	GTA	TGG	ATG	GGC	AAA	TGG	1068
	Arg	Gln	Val	Gly	Lys	Gly	Arg	Tyr	Gly	Glu	Val	Trp	Met	Gly	Lys	Trp	
			240				245						250				
60	CGT	GGC	GAA	AAA	GTG	GCG	GTG	AAA	GTA	TTC	TTT	ACC	ACT	GAA	GAA	GCC	1116
	Arg	Gly	Glu	Lys	Val	Ala	Val	Lys	Val	Phe	Phe	Thr	Thr	Glu	Glu	Ala	
			255				260					265					
65	AGC	TGG	TTT	CGA	GAA	ACA	GAA	ATC	TAC	CAA	ACT	GTG	CTA	ATG	CGC	CAT	1164
	Ser	Trp	Phe	Arg	Glu	Thr	Glu	Ile	Tyr	Gln	Thr	Val	Leu	Met	Arg	His	
	270					275					280				285		
70	GAA	AAC	ATA	CTT	GGT	TTC	ATA	GCG	GCA	GAC	ATT	AAA	GGT	ACA	GGT	TCC	1212
	Glu	Asn	Ile	Leu	Gly	Phe	Ile	Ala	Ala	Asp	Ile	Lys	Gly	Thr	Gly	Ser	
					290					295					300		
75	TGG	ACT	CAG	CTC	TAT	TTG	ATT	ACT	GAT	TAC	CAT	GAA	AAT	GGA	TCT	CTC	1260

[illegible]

5 AACTTGGTTC TCAGACTCTT TCTTCACTAC GTGTTACACAG GCTGCTAATA TTAAACCTTT 2055
 CAGTACTCTT ATTAGGATAC AAGCTGGGAA CTTCTAAACA CTTCAATCTT TATATATGGA 2115
 CAGCTTTATT TTAAATGTGG TTTTGTATGC CTTTTTTTAA GTGGGTTTTT ATGAACTGCA 2175
 TCAAGACTTC AATCCTGATT AGTGTCTCCA GTCAAGCTCT GGGTACTGAA TTGCCTGTTC 2235
 10 ATAAAACGGT GCTTTCTGTG AAAGCCTTAA GAAGATAAAT GAGCGCAGCA GAGATGGAGA 2295
 AATAGACTTT GCCTTTTACC TGAGACATTC AGTTCGTTTG TATTCTACCT TTGTAAAACA 2355
 GCCTATAGAT GATGATGTGT TTGGGATACT GCTTATTTTA TGATAGTTTG TCCTGTGTCC 2415
 15 TTAGTGATGT GTGTGTGTCT CCATGCACAT GCACGCCGGG ATTCCTCTGC TGCCATTGTA 2475
 ATTAGAAGAA AATAATTTAT ATGCATGCAC AGGAAGATAT TGGTGGCCGG TGGTTTTGTG 2535
 20 CTTTAAAAAT GCAATATCTG ACCAAGATTC GCCAATCTCA TACAAGCCAT TACTTTTGCA 2595
 AGTGAGATAG CTTCCCCACC AGCTTTATTT TTAAACATGA AAGCTGATGC CAAGGCCAAA 2655
 AGAAGTTTAA AGCATCTGTA AATTGGACT GTTTTCCTTC AACCACCATT TTTTTGTGG 2715
 25 TTATTATTTT TGTCACGGAA AGCATCCTCT CCAAAGTTGG AGCTTCTATT GCCATGAACC 2775
 ATGCTTACAA AGAAAGCACT TCTTATTGAA GTGAATTCCT GCATTTGATA GCAATGTAAG 2835
 30 TGCCTATAAC CATGTTCTAT ATTCTTTATT CTCAGTAACT TTTAAAAGGG AAGTTATTTA 2895
 TATTTTGTGT ATAATGTGCT TTATTTGCAA ATCACCC 2932

35 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 532 amino acids
 (B) TYPE: amino acid
 40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

45 Met Thr Gln Leu Tyr Ile Tyr Ile Arg Leu Leu Gly Ala Tyr Leu Phe
 1 5 10 15
 Ile Ile Ser Arg Val Gln Gly Gln Asn Leu Asp Ser Met Leu His Gly
 50 20 25 30
 Thr Gly Met Lys Ser Asp Ser Asp Gln Lys Lys Ser Glu Asn Gly Val
 35 40 45
 55 Thr Leu Ala Pro Glu Asp Thr Leu Pro Phe Leu Lys Cys Tyr Cys Ser
 50 55 60
 Gly His Cys Pro Asp Asp Ala Ile Asn Asn Thr Cys Ile Thr Asn Gly
 60 65 70 75 80

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Arg Val Gly Thr Lys Arg Tyr Met Ala Pro Glu Val Leu Asp Glu Ser
 405 410 415
 5 Leu Asn Lys Asn His Phe Gln Pro Tyr Ile Met Ala Asp Ile Tyr Ser
 420 425 430
 Phe Gly Leu Ile Ile Trp Glu Met Ala Arg Arg Cys Ile Thr Gly Gly
 435 440 445
 10 Ile Val Glu Glu Tyr Gln Leu Pro Tyr Tyr Asn Met Val Pro Ser Asp
 450 455 460
 Pro Ser Tyr Glu Asp Met Arg Glu Val Val Cys Val Lys Arg Leu Arg
 465 470 475 480
 15 Pro Ile Val Ser Asn Arg Trp Asn Ser Asp Glu Cys Leu Arg Ala Val
 485 490 495
 Leu Lys Leu Met Ser Glu Cys Trp Ala His Asn Pro Ala Ser Arg Leu
 500 505 510
 Thr Ala Leu Arg Ile Lys Lys Thr Leu Ala Lys Met Val Glu Ser Gln
 515 520 525
 25 Asp Val Lys Ile
 530

(2) INFORMATION FOR SEQ ID NO:7:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1952 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 35 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: cDNA
 40 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 187..1695
 (D) OTHER INFORMATION: /product= "Murine ALK6"
 45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AAGCGGCGGC AGAAGTTGCC GGCCTGGTGC TCGTAGTGAG GGCGCGGAGG ACCCGGGACC 60
 50 TGGGAAGCGG CGGCGGGTTA ACTTCGGCTG AATCACAACC ATTTGGCGCT GAGCTATGAC 120
 AAGAGAGCAA ACAAAAAGTT AAAGGAGCAA CCCGGCCATA AGTGAAGAGA GAAGTTTATT 180
 GATAAC ATG CTC TTA CGA AGC TCT GGA AAA TTA AAT GTG GGC ACC AAG 228
 55 Met Leu Leu Arg Ser Ser Gly Lys Leu Asn Val Gly Thr Lys
 1 5 10
 AAG GAG GAT GGA GAG AGT ACA GCC CCC ACC CCT CGG CCC AAG ATC CTA 276
 60 Lys Glu Asp Gly Glu Ser Thr Ala Pro Thr Pro Arg Pro Lys Ile Leu
 15 20 25 30

	CGT	TGT	AAA	TGC	CAC	CAC	CAC	TGT	CCG	GAA	GAC	TCA	GTC	AAC	AAT	ATC	324
	Arg	Cys	Lys	Cys	His	His	His	Cys	Pro	Glu	Asp	Ser	Val	Asn	Asn	Ile	
					35					40					45		
5	TGC	AGC	ACA	GAT	GGG	TAC	TGC	TTC	ACG	ATG	ATA	GAA	GAA	GAT	GAC	TCT	372
	Cys	Ser	Thr	Asp	Gly	Tyr	Cys	Phe	Thr	Met	Ile	Glu	Glu	Asp	Asp	Ser	
				50					55					60			
10	GGA	ATG	CCT	GTT	GTC	ACC	TCT	GGA	TGT	CTA	GGA	CTA	GAA	GGG	TCA	GAT	420
	Gly	Met	Pro	Val	Val	Thr	Ser	Gly	Cys	Leu	Gly	Leu	Glu	Gly	Ser	Asp	
			65					70					75				
15	TTT	CAA	TGT	CGT	GAC	ACT	CCC	ATT	CCT	CAT	CAA	AGA	AGA	TCA	ATT	GAA	468
	Phe	Gln	Cys	Arg	Asp	Thr	Pro	Ile	Pro	His	Gln	Arg	Arg	Ser	Ile	Glu	
		80					85					90					
20	TGC	TGC	ACA	GAA	AGG	AAT	GAG	TGT	AAT	AAA	GAC	CTC	CAC	CCC	ACT	CTG	516
	Cys	Cys	Thr	Glu	Arg	Asn	Glu	Cys	Asn	Lys	Asp	Leu	His	Pro	Thr	Leu	
	95					100					105				110		
	CCT	CCT	CTC	AAG	GAC	AGA	GAT	TTT	GTT	GAT	GGG	CCC	ATA	CAC	CAC	AAG	564
	Pro	Pro	Leu	Lys	Asp	Arg	Asp	Phe	Val	Asp	Gly	Pro	Ile	His	His	Lys	
					115					120					125		
25	GCC	TTG	CTT	ATC	TCT	GTG	ACT	GTC	TGT	AGT	TTA	CTC	TTG	GTC	CTC	ATT	612
	Ala	Leu	Leu	Ile	Ser	Val	Thr	Val	Cys	Ser	Leu	Leu	Leu	Val	Leu	Ile	
				130					135					140			
30	ATT	TTA	TTC	TGT	TAC	TTC	AGG	TAT	AAA	AGA	CAA	GAA	GCC	CGA	CCT	CGG	660
	Ile	Leu	Phe	Cys	Tyr	Phe	Arg	Tyr	Lys	Arg	Gln	Glu	Ala	Arg	Pro	Arg	
			145					150					155				
35	TAC	AGC	ATT	GGG	CTG	GAG	CAG	GAC	GAG	ACA	TAC	ATT	CCT	CCT	GGA	GAG	708
	Tyr	Ser	Ile	Gly	Leu	Glu	Gln	Asp	Glu	Thr	Tyr	Ile	Pro	Pro	Gly	Glu	
		160					165					170					
40	TCC	CTG	AGA	GAC	TTG	ATC	GAG	CAG	TCT	CAG	AGC	TCG	GGA	AGT	GGA	TCA	756
	Ser	Leu	Arg	Asp	Leu	Ile	Glu	Gln	Ser	Gln	Ser	Ser	Gly	Ser	Gly	Ser	
	175					180					185					190	
	GGC	CTC	CCT	CTG	CTG	GTC	CAA	AGG	ACA	ATA	GCT	AAG	CAA	ATT	CAG	ATG	804
	Gly	Leu	Pro	Leu	Leu	Val	Gln	Arg	Thr	Ile	Ala	Lys	Gln	Ile	Gln	Met	
					195					200					205		
45	GTG	AAG	CAG	ATT	GGA	AAA	GGC	CGC	TAT	GGC	GAG	GTG	TGG	ATG	GGA	AAG	852
	Val	Lys	Gln	Ile	Gly	Lys	Gly	Arg	Tyr	Gly	Glu	Val	Trp	Met	Gly	Lys	
			210					215					220				
50	TGG	CGT	GGA	GAA	AAG	GTG	GCT	GTG	AAA	GTG	TTC	TTC	ACC	ACG	GAG	GAA	900
	Trp	Arg	Gly	Glu	Lys	Val	Ala	Val	Lys	Val	Phe	Phe	Thr	Thr	Glu	Glu	
			225					230					235				
55	GCC	AGC	TGG	TTC	CGA	GAG	ACT	GAG	ATA	TAT	CAG	ACG	GTC	CTG	ATG	CGG	948
	Ala	Ser	Trp	Phe	Arg	Glu	Thr	Glu	Ile	Tyr	Gln	Thr	Val	Leu	Met	Arg	
		240					245					250					
60	CAT	GAG	AAT	ATT	CTG	GGG	TTC	ATT	GCT	GCA	GAT	ATC	AAA	GGG	ACT	GGG	996
	His	Glu	Asn	Ile	Leu	Gly	Phe	Ile	Ala	Ala	Asp	Ile	Lys	Gly	Thr	Gly	
	255					260					265					270	

	TCC	TGG	ACT	CAG	TTG	TAC	CTC	ATC	ACA	GAC	TAT	CAT	GAA	AAC	GGC	TCC	1044
	Ser	Trp	Thr	Gln	Leu	Tyr	Leu	Ile	Thr	Asp	Tyr	His	Glu	Asn	Gly	Ser	
5				275						280					285		
	CTT	TAT	GAC	TAT	CTG	AAA	TCC	ACC	ACC	TTA	GAC	GCA	AAG	TCC	ATG	CTG	1092
	Leu	Tyr	Asp	Tyr	Leu	Lys	Ser	Thr	Thr	Leu	Asp	Ala	Lys	Ser	Met	Leu	
				290					295					300			
10	AAG	CTA	GCC	TAC	TCC	TCT	GTC	AGC	GGC	CTA	TGC	CAT	TTA	CAC	ACG	GAA	1140
	Lys	Leu	Ala	Tyr	Ser	Ser	Val	Ser	Gly	Leu	Cys	His	Leu	His	Thr	Glu	
			305					310					315				
15	ATC	TTT	AGC	ACT	CAA	GGC	AAG	CCA	GCA	ATC	GCC	CAT	CGA	GAC	TTG	AAA	1188
	Ile	Phe	Ser	Thr	Gln	Gly	Lys	Pro	Ala	Ile	Ala	His	Arg	Asp	Leu	Lys	
		320					325					330					
20	AGT	AAA	AAC	ATC	CTG	GTG	AAG	AAA	AAT	GGA	ACT	TGC	TGC	ATA	GCA	GAC	1236
	Ser	Lys	Asn	Ile	Leu	Val	Lys	Lys	Asn	Gly	Thr	Cys	Cys	Ile	Ala	Asp	
	335					340					345					350	
25	CTG	GGC	TTG	GCT	GTC	AAG	TTC	ATT	AGT	GAC	ACA	AAT	GAG	GTT	GAC	ATC	1284
	Leu	Gly	Leu	Ala	Val	Lys	Phe	Ile	Ser	Asp	Thr	Asn	Glu	Val	Asp	Ile	
				355						360					365		
	CCA	CCC	AAC	ACC	CGG	GTT	GGC	ACC	AAG	CGC	TAT	ATG	CCT	CCA	GAA	GTG	1332
	Pro	Pro	Asn	Thr	Arg	Val	Gly	Thr	Lys	Arg	Tyr	Met	Pro	Pro	Glu	Val	
				370					375					380			
30	CTG	GAC	GAG	AGC	TTG	AAT	AGA	AAC	CAT	TTC	CAG	TCC	TAC	ATT	ATG	GCT	1380
	Leu	Asp	Glu	Ser	Leu	Asn	Arg	Asn	His	Phe	Gln	Ser	Tyr	Ile	Met	Ala	
			385					390					395				
35	GAC	ATG	TAC	AGC	TTT	GGA	CTC	ATC	CTC	TGG	GAG	ATT	GCA	AGG	AGA	TGT	1428
	Asp	Met	Tyr	Ser	Phe	Gly	Leu	Ile	Leu	Trp	Glu	Ile	Ala	Arg	Arg	Cys	
		400					405					410					
40	GTT	TCT	GGA	GGT	ATA	GTG	GAA	GAA	TAC	CAG	CTT	CCC	TAT	CAC	GAC	CTG	1476
	Val	Ser	Gly	Gly	Ile	Val	Glu	Glu	Tyr	Gln	Leu	Pro	Tyr	His	Asp	Leu	
	415					420					425					430	
45	GTG	CCC	AGT	GAC	CCT	TCT	TAT	GAG	GAC	ATG	AGA	GAA	ATT	GTG	TGC	ATG	1524
	Val	Pro	Ser	Asp	Pro	Ser	Tyr	Glu	Asp	Met	Arg	Glu	Ile	Val	Cys	Met	
					435				440						445		
	AAG	AAG	TTA	CGG	CCT	TCA	TTC	CCC	AAT	CGA	TGG	AGC	AGT	GAT	GAG	TGT	1572
	Lys	Lys	Leu	Arg	Pro	Ser	Phe	Pro	Asn	Arg	Trp	Ser	Ser	Asp	Glu	Cys	
				450					455					460			
50	CTC	AGG	CAG	ATG	GGG	AAG	CTT	ATG	ACA	GAG	TGC	TGG	GCG	CAG	AAT	CCT	1620
	Leu	Arg	Gln	Met	Gly	Lys	Leu	Met	Thr	Glu	Cys						

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ATTTACACAGA AGCATCGTTA GCCCAAGCCT TGAACGTTAG CCTACTGCCC AGTGAGTTCA 1782
 GACTTTCCTG GAAGAGAGCA CGGTGGGCAG ACACAGAGGA ACCCAGAAAC ACGGATTCAT 1842
 5 CATGGCTTTC TGAGGAGGAG AACTGTTTG GGTAACCTGT TCAAGATATG ATGCATGTTG 1902
 CTTTCTAAGA AAGCCCTGTA TTTTGAATTA CCATTTTTTT ATAAAAAAA 1952

10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 502 amino acids
 15 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Leu Leu Arg Ser Ser Gly Lys Leu Asn Val Gly Thr Lys Lys Glu
 1 5 10 15
 25 Asp Gly Glu Ser Thr Ala Pro Thr Pro Arg Pro Lys Ile Leu Arg Cys
 20 25 30
 Lys Cys His His His Cys Pro Glu Asp Ser Val Asn Asn Ile Cys Ser
 35 40 45
 30 Thr Asp Gly Tyr Cys Phe Thr Met Ile Glu Glu Asp Asp Ser Gly Met
 50 55 60
 Pro Val Val Thr Ser Gly Cys Leu Gly Leu Glu Gly Ser Asp Phe Gln
 35 65 70 75 80
 Cys Arg Asp Thr Pro Ile Pro His Gln Arg Arg Ser Ile Glu Cys Cys
 85 90 95
 40 Thr Glu Arg Asn Glu Cys Asn Lys Asp Leu His Pro Thr Leu Pro Pro
 100 105 110
 Leu Lys Asp Arg Asp Phe Val Asp Gly Pro Ile His His Lys Ala Leu
 115 120 125
 45 Leu Ile Ser Val Thr Val Cys Ser Leu Leu Leu Val Leu Ile Ile Leu
 130 135 140
 Phe Cys Tyr Phe Arg Tyr Lys Arg Gln Glu Ala Arg Pro Arg Tyr Ser
 50 145 150 155 160
 Ile Gly Leu Glu Gln Asp Glu Thr Tyr Ile Pro Pro Gly Glu Ser Leu
 165 170 175
 55 Arg Asp Leu Ile Glu Gln Ser Gln Ser Ser Gly Ser Gly Ser Gly Leu
 180 185 190
 Pro Leu Leu Val Gln Arg Thr Ile Ala Lys Gln Ile Gln Met Val Lys
 195 200 205
 60

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Gln Ile Gly Lys Gly Arg Tyr Gly Glu Val Trp Met Gly Lys Trp Arg
 210 215 220
 5 Gly Glu Lys Val Ala Val Lys Val Phe Phe Thr Thr Glu Glu Ala Ser
 225 230 235 240
 Trp Phe Arg Glu Thr Glu Ile Tyr Gln Thr Val Leu Met Arg His Glu
 245 250 255
 10 Asn Ile Leu Gly Phe Ile Ala Ala Asp Ile Lys Gly Thr Gly Ser Trp
 260 265 270
 Thr Gln Leu Tyr Leu Ile Thr Asp Tyr His Glu Asn Gly Ser Leu Tyr
 275 280 285
 15 Asp Tyr Leu Lys Ser Thr Thr Leu Asp Ala Lys Ser Met Leu Lys Leu
 290 295 300
 20 Ala Tyr Ser Ser Val Ser Gly Leu Cys His Leu His Thr Glu Ile Phe
 305 310 315 320
 Ser Thr Gln Gly Lys Pro Ala Ile Ala His Arg Asp Leu Lys Ser Lys
 325 330 335
 25 Asn Ile Leu Val Lys Lys Asn Gly Thr Cys Cys Ile Ala Asp Leu Gly
 340 345 350
 Leu Ala Val Lys Phe Ile Ser Asp Thr Asn Glu Val Asp Ile Pro Pro
 355 360 365
 30 Asn Thr Arg Val Gly Thr Lys Arg Tyr Met Pro Pro Glu Val Leu Asp
 370 375 380
 35 Glu Ser Leu Asn Arg Asn His Phe Gln Ser Tyr Ile Met Ala Asp Met
 385 390 395 400
 Tyr Ser Phe Gly Leu Ile Leu Trp Glu Ile Ala Arg Arg Cys Val Ser
 405 410 415
 40 Gly Gly Ile Val Glu Glu Tyr Gln Leu Pro Tyr His Asp Leu Val Pro
 420 425 430
 Ser Asp Pro Ser Tyr Glu Asp Met Arg Glu Ile Val Cys Met Lys Lys
 435 440 445
 45 Leu Arg Pro Ser Phe Pro Asn Arg Trp Ser Ser Asp Glu Cys Leu Arg
 450 455 460
 50 Gln Met Gly Lys Leu Met Thr Glu Cys Trp Ala Gln Asn Pro Ala Ser
 465 470 475 480
 Arg Leu Thr Ala Leu Arg Val Lys Lys Thr Leu Ala Lys Met Ser Glu
 485 490 495
 55 Ser Gln Asp Ile Lys Leu
 500

(2) INFORMATION FOR SEQ ID NO:9:

60

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1822 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 49..1341
 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "OP1"
 /evidence= EXPERIMENTAL
 /standard_name= "OP1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

20	GGTGCGGGCC CGGAGCCCGG AGCCCGGGTA GCGCGTAGAG CCGGCGCG ATG CAC GTG	57
	Met His Val	
	1	
25	CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA	105
	Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala	
	5 10 15	
30	CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC	153
	Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn	
	20 25 30 35	
35	GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG	201
	Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg	
	40 45 50	
40	CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC	249
	Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg	
	55 60 65	
45	CCG CGC CCG CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG	297
	Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met	
	70 75 80	
50	CTG GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGC CCC GGC	345
	Leu Asp Leu Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Gly Pro Gly	
	85 90 95	
55	GGC CAG GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC	393
	Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly	
	100 105 110 115	
60	CCC CCT CTG GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC	441
	Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp	
	120 125 130	
65	ATG GTC ATG AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC	489
	Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe	
	135 140 145	
70	CAC CCA CGC TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC	537

	His	Pro	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	Ser	Lys	Ile	
			150					155					160				
5	CCA	GAA	GGG	GAA	GCT	GTC	ACG	GCA	GCC	GAA	TTC	CGG	ATC	TAC	AAG	GAC	585
	Pro	Glu	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Asp	
		165					170					175					
10	TAC	ATC	CGG	GAA	CGC	TTC	GAC	AAT	GAG	ACG	TTC	CGG	ATC	AGC	GTT	TAT	633
	Tyr	Ile	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Arg	Ile	Ser	Val	Tyr	
	180					185					190					195	
15	CAG	GTG	CTC	CAG	GAG	CAC	TTG	GGC	AGG	GAA	TCG	GAT	CTC	TTC	CTG	CTC	681
	Gln	Val	Leu	Gln	Glu	His	Leu	Gly	Arg	Glu	Ser	Asp	Leu	Phe	Leu	Leu	
				200						205					210		
	GAC	AGC	CGT	ACC	CTC	TGG	GCC	TCG	GAG	GAG	GGC	TGG	CTG	GTG	TTT	GAC	729
	Asp	Ser	Arg	Thr	Leu	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Val	Phe	Asp	
				215					220					225			
20	ATC	ACA	GCC	ACC	AGC	AAC	CAC	TGG	GTG	GTC	AAT	CCG	CGG	CAC	AAC	CTG	777
	Ile	Thr	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His	Asn	Leu	
			230					235					240				
25	GGC	CTG	CAG	CTC	TCG	GTG	GAG	ACG	CTG	GAT	GGG	CAG	AGC	ATC	AAC	CCC	825
	Gly	Leu	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	Ile	Asn	Pro	
		245					250					255					
30	AAG	TTG	GCG	GGC	CTG	ATT	GGG	CGG	CAC	GGG	CCC	CAG	AAC	AAG	CAG	CCC	873
	Lys	Leu	Ala	Gly	Leu	Ile	Gly	Arg	His	Gly	Pro	Gln	Asn	Lys	Gln	Pro	
		260				265					270					275	
35	TTC	ATG	GTG	GCT	TTC	TTC	AAG	GCC	ACG	GAG	GTC	CAC	TTC	CGC	AGC	ATC	921
	Phe	Met	Val	Ala	Phe	Phe	Lys	Ala	Thr	Glu	Val	His	Phe	Arg	Ser	Ile	
				280						285					290		
	CGG	TCC	ACG	GGG	AGC	AAA	CAG	CGC	AGC	CAG	AAC	CGC	TCC	AAG	ACG	CCC	969
	Arg	Ser	Thr	Ser	Lys	Gln	Arg	Ser	Gln	Asn	Arg	Ser	Lys	Thr	Pro		
				295				300					305				
40	AAG	AAC	CAG	GAA	GCC	CTG	CGG	ATG	GCC	AAC	GTG	GCA	GAG	AAC	AGC	AGC	1017
	Lys	Asn	Gln	Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala	Glu	Asn	Ser	Ser	
			310					315					320				
45	AGC	GAC	CAG	AGG	CAG	GCC	TGT	AAG	AAG	CAC	GAG	CTG	TAT	GTC	AGC	TTC	1065
	Ser	Asp	Gln	Arg	Gln	Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val	Ser	Phe	
		325				330					335						
50	CGA	GAC	CTG	GGC	TGG	CAG	GAC	TGG	ATC	ATC	GCG	CCT	GAA	GGC	TAC	GCC	1113
	Arg	Asp	Leu	Gly	Trp	Gln	Asp	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala	
		340				345					350					355	
55	GCC	TAC	TAC	TGT	GAG	GGG	GAG	TGT	GCC	TTC	CCT	CTG	AAC	TCC	TAC	ATG	1161
	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asn	Ser	Tyr	Met	
				360					365						370		
	AAC	GCC	ACC	AAC	CAC	GCC	ATC	GTG	CAG	ACG	CTG	GTC	CAC	TTC	ATC	AAC	1209
	Asn	Ala	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	His	Phe	Ile	Asn	
				375				380						385			
60	CCG	GAA	ACG	GTG	CCC	AAG	CCC	TGC	TGT	GCG	CCC	ACG	CAG	CTC	AAT	GCC	1257

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Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala
      390                      395                      400
5  ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA 1305
   Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys
      405                      410                      415

TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC 1351
Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
10 420                      425                      430

GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG 1411
15 GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG 1471
   TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC 1531
   ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC 1591
20 GCATAAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT 1651
   CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG 1711
   GCGGTGGCAA GGGGTGGGCA CATTTGGTGTG TGTGCGAAAG GAAAATTGAC CCGGAAGTTC 1771
25 CTGTAATAAAA TGTCACAATA AAACGAATGA ATGAAAAAAA AAAAAAAAAA A 1822

```

(2) INFORMATION FOR SEQ ID NO:10:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 431 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```

40 Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
   1          5          10          15

Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
   20          25          30

45 Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
   35          40          45

50 Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
   50          55          60

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
   65          70          75          80

55 Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly
   85          90          95

Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
   100          105          110
60

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Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
 115 120 125
 5 Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
 130 135 140
 Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu
 145 150 155 160
 10 Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
 165 170 175
 Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
 180 185 190
 15 Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
 195 200 205
 Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
 210 215 220
 20 Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
 225 230 235 240
 25 His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
 245 250 255
 Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
 260 265 270
 30 Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
 275 280 285
 Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
 290 295 300
 Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
 305 310 315 320
 40 Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
 325 330 335
 Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
 340 345 350
 45 Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
 355 360 365
 Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
 370 375 380
 Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
 385 390 395 400
 55 Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
 405 410 415
 Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 420 425 430
 60

- 82 -

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- 10 (A) NAME/KEY: Protein
 (B) LOCATION: 1..102
 15 (D) OTHER INFORMATION: /label= OPX
 /note= "Each Xaa is independently selected from
 a group of one or more specified amino acids
 as defined in the specification"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

20 Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa
 1 5 10 15
 25 Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
 20 25 30
 Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
 35 40 45
 30 Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
 50 55 60
 35 Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
 65 70 75 80
 Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
 85 90 95
 40 Xaa Ala Cys Gly Cys His
 100

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 28 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 50 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

55 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

60

GCGGATCCTG TTGTGAAGGN AATATGTG

28

(2) INFORMATION FOR SEQ ID NO:13:

- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

15 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

20 GCGATCCGTC GCAGTCAAAA TTTT

24

(2) INFORMATION FOR SEQ ID NO:14:

- 25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

35 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

40 GCGGATCCGC GATATATTAA AAGCAA

26

(2) INFORMATION FOR SEQ ID NO:15:

- 45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

55 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

60 CGGAATTCTG GTGCCATATA

20

What is claimed is:

- 1 1. A method for identifying an OP-1 receptor-binding analog, said
2 analog being characterized as having substantially the same
3 binding affinity for a cell surface receptor as OP-1, the method
4 comprising the steps of:
 - 5 (a) providing a sample containing a protein selected from the group
6 consisting of:
 - 7 (i) a polypeptide chain comprising an amino acid sequence
8 defined by residues 16-123 of Seq. ID No. 3 (ALK-2), or an
9 OP1-binding analog thereof;
 - 10 (ii) a polypeptide chain comprising an amino acid sequence
11 defined by residues 24-152 of Seq. ID No. 5 (ALK-3),, or an
12 OP1-binding analog thereof;
 - 13 (iii) a polypeptide chain comprising an amino acid sequence
14 defined by residues 23-122 of Seq. ID No. 7 (ALK-6),, or an
15 OP1 binding analog thereof;
 - 16 (iv) a polypeptide chain having binding affinity for OP-1 and
17 sharing at least 40% amino acid identity with residues 23-
18 122 of Seq. ID No. 7 (ALK-6),;
 - 19 (v) a polypeptide chain having binding affinity for OP-1 and
20 encoded by a nucleic acid obtainable by amplification with
21 one or more primer sequences defined by Seq. ID Nos. 12-15;
22 or
 - 23 (vi) a polypeptide chain having binding affinity for OP-1 and
24 encoded by a nucleic acid that hybridizes under stringent
25 conditions with a nucleic acid comprising the sequence
26 defined by nucleotides 256-552 of Seq. ID No. 7 (ALK-6),;
 - 27 (b) contacting said sample with a candidate OP1 receptor- binding
28 analog; and
 - 29 (c) detecting specific binding between said candidate OP1 receptor-
30 binding analog and said protein.
- 1 2. A method for identifying an OP-1 receptor-binding analog, said
2 analog being characterized as having substantially the same
3 binding affinity for a cell surface receptor as OP1, the method
4 comprising the steps of:

- 5 (a) providing a cell that expresses a surface receptor protein having
6 binding specificity for OP-1 selected from the group consisting
7 of:
- 8 (i) a polypeptide chain comprising an amino acid sequence
9 defined by residues 16-123 of Seq. ID No. 3 (ALK-2), or an
10 OP1-binding analog thereof;
- 11 (ii) a polypeptide chain comprising an amino acid sequence
12 defined by residues 24-152 of Seq. ID No. 5 (ALK-3),, or an
13 OP1-binding analog thereof;
- 14 (iii) a polypeptide chain comprising an amino acid sequence
15 defined by residues 23-122 of Seq. ID No. 7 (ALK-6),, or an
16 OP1 binding analog thereof;
- 17 (iv) a polypeptide chain having binding affinity for OP-1 and
18 sharing at least 40% amino acid identity with residues 23-
19 122 of Seq. ID No. 7 (ALK-6),;
- 20 (v) a polypeptide chain having binding affinity for OP-1 and
21 encoded by a nucleic acid obtainable by amplification with
22 one or more primer sequences defined by Seq. ID Nos. 12-15;
23 or
- 24 (vi) a polypeptide chain having binding affinity for OP-1 and
25 encoded by a nucleic acid that hybridizes under stringent
26 conditions with a nucleic acid comprising the sequence
27 defined by nucleotides 256-552 of Seq. ID No. 7 (ALK-6),;
- 28 (b) contacting said cell with a candidate OP1 receptor-binding
29 analog; and
- 30 (c) detecting induction of an OP-1-mediated cellular response.
- 1 3. The method of claim 2 wherein said OP-1 mediated cellular response
2 detected in step (c) is induction of a kinase activity, inhibition of
3 epithelial cell growth, or induction of a cell differentiation
4 marker.
- 1 4. The method of claim 2 or 3 wherein said cell comprises a transfected
2 nucleic acid comprising a reporter gene in operative association with a
3 control element derived from an OP-1 inducible protein.
- 1 5. The method of any of claims 1-4 wherein said sample further comprises
2 part or all of a Type II serine/threonine kinase receptor protein
3 having binding affinity for OP-1, activin or BMP-4.

- 1 6. A method for producing an OP-1 receptor binding analog, the method
2 comprising the steps of:
- 3 (a) obtaining, by the method of any of claims 1-5, a candidate OP-1
4 binding analog, and
- 5 (b) producing either said candidate analog or a second OP-1 binding
6 analog derived from said candidate and having substantially the
7 same OP-1 receptor-binding domain as said candidate.
- 1 7. The method of producing an OP-1 receptor-binding analog of claim 6
2 wherein said analog produced in step (b) is by recombinant DNA
3 techniques, or by nonbiological peptide synthesis.
- 1 8. A kit for identifying OP-1 or a candidate OP-1 receptor binding analog
2 in a sample, the kit comprising:
- 3 (a) a receptacle adapted to receive a sample and containing a protein
4 selected from the group consisting of:
- 5 (i) a polypeptide chain comprising an amino acid sequence
6 defined by residues 16-123 of Seq. ID No. 3 (ALK-2), or an
7 OP1-binding analog thereof;
- 8 (ii) a polypeptide chain comprising an amino acid sequence
9 defined by residues 24-152 of Seq. ID No. 5 (ALK-3),, or an
10 OP1-binding analog thereof;
- 11 (iii) a polypeptide chain comprising an amino acid sequence
12 defined by residues 23-122 of Seq. ID No. 7 (ALK-6),, or an
13 OP1 binding analog thereof;
- 14 (iv) a polypeptide chain having binding affinity for OP-1 and
15 sharing at least 40% amino acid identity with residues 23-
16 122 of Seq. ID No. 7 (ALK-6),;
- 17 (v) a polypeptide chain having binding affinity for OP-1 and
18 encoded by a nucleic acid obtainable by amplification with
19 one or more primer sequences defined by Seq. ID Nos. 12-15;
20 or
- 21 (vi) a polypeptide chain having binding affinity for OP-1 and
22 encoded by a nucleic acid that hybridizes under stringent
23 conditions with a nucleic acid comprising the sequence
24 defined by nucleotides 256-552 of Seq. ID No. 7 (ALK-6),;
25 and
- 26 (b) means for detecting interaction of OP-1 or a candidate OP-1
27 receptor-binding analog with said protein of part (a), said OP-1

- 28 or candidate analog comprising part of said sample provided to
29 said receptacle.
- 1 9. The kit of claim 8 wherein said means in part (b) comprises either
2 (i) means for detecting specific binding interaction of OP-1
3 or said candidate analog with said protein; or
4 (ii) means for detecting induction of an OP-1 mediated cellular
5 response.
- 1 10. The kit of claim 8 or 9 further comprising a serine/threonine Type II
2 receptor having binding specificity for OP-1, activin or BMP-4.
- 1 11. An OP-1 receptor-binding analog produced by the method of any of claims
2 1-7 or use of the kit of claims 8-10.
- 1 12. The analog produced by the method of any of claims 1-8, said analog
2 (i) comprising an amino acid sequence sharing greater than 60%
3 identity with the C-terminal 96 amino acids of the sequence
4 represented by Seq. ID No. 9 (OP-1, residues 335-431), and
5 (ii) being substantially incapable of inducing an OP-1 mediated
6 cellular response.
- 1 13. The analog of claim 11 or 12 further having binding affinity for a
2 Type II serine/threonine kinase cell surface receptor.
- 1 14. The analog of claim 13 wherein said Type II receptor also has binding
2 affinity for activin or BMP-4.
- 1 15. An isolated ligand-receptor complex comprising two molecules
2 interacting as specific binding partners, the first said molecule
3 defining said ligand and comprising at least the C-terminal 96 amino
4 acids of OP1 (residues 335-431 of Seq ID No. 9) or a receptor-binding
5 analog thereof, and the second said molecule defining said receptor and
6 being selected from the group consisting of:
7 (i) a polypeptide chain comprising an amino acid sequence
8 defined by residues 16-123 of Seq. ID No. 3 (ALK-2), or an
9 OP1-binding analog thereof;
10 (ii) a polypeptide chain comprising an amino acid sequence
11 defined by residues 24-152 of Seq. ID No. 5 (ALK-3), or an
12 OP1-binding analog thereof;
13 (iii) a polypeptide chain comprising an amino acid sequence
14 defined by residues 23-122 of Seq. ID No. 7 (ALK-6), or an
15 OP1 binding analog thereof;

- 16 (iv) a polypeptide chain having binding affinity for OP-1 and
17 sharing at least 40% amino acid identity with residues 23-
18 122 of Seq. ID No. 7 (ALK-6).;
- 19 (v) a polypeptide chain having binding affinity for OP-1 and
20 encoded by a nucleic acid obtainable by amplification with
21 one or more primer sequences defined by Seq. ID Nos. 12-15;
22 or
- 23 (vi) a polypeptide chain having binding affinity for OP-1 and
24 encoded by a nucleic acid that hybridizes under stringent
25 conditions with a nucleic acid comprising the sequence
26 defined by nucleotides 256-552 of Seq. ID No. 7 (ALK-6),.
- 1 16. The complex of claim 15 further comprising part or all of a Type II
2 serine/threonine kinase receptor .
- 1 17. The complex of claim 16 wherein said Type II receptor also has binding
2 affinity for activin or BMP-4.
- 1 18. The complex of any of claims 15-17 wherein said first molecule defining
2 said ligand is an OP-1 receptor-binding analog comprises part or all
3 of the proteins selected from the group consisting of 60A, BMP-5, BMP-
4 6, Vgr-1, OP2, OP3 and receptor-binding amino acid sequence variants or
5 xenogenic homologs thereof.
- 1 19. An isolated binding partner having specific binding affinity for an
2 epitope on a ligand-receptor complex, said complex being characterized
3 as comprising an OP-1 protein or an analog thereof in specific binding
4 interaction with the ligand binding domain of a cell surface receptor
5 defined by Seq. ID No. 3 (ALK-2), 5, or 7, or an OP1-binding analog
6 thereof; said binding partner having substantially no binding affinity
7 for the uncomplexed form of said OP-1 protein or OP-1 protein analog.
- 1 20. The isolated binding partner of claim 19 wherein said binding partner
2 is further characterized as having substantially no binding affinity
3 for the uncomplexed form of said cell surface receptor protein or said
4 analog thereof.
- 1 21. The binding partner of claim 19 wherein said binding partner is a
2 monoclonal or polyclonal antibody.
- 1 22. Use of the OP-1 receptor-binding analog of any claims 11-14 in a method
2 for
- 3 (i) antagonizing OP-1 binding to a cell surface receptor; or
4 (ii) antagonizing induction of an OP-1 mediated cellular
5 response.

- 1 23. The use according to claim 22 wherein said OP-1 receptor-binding analog
2 comprises an antibody having binding specificity for
- 3 (i) the ligand binding domain of a cell surface receptor defined
4 by Seq. ID Nos. 3, 5, or 7 or an OP-1 binding analog
5 thereof; or
- 6 (ii) the receptor binding domain of OP-1, represented by Seq. ID
7 No. 9, or a receptor-binding analog thereof.
- 1 24. Use of a protein selected from the group consisting of:
- 2 (i) a polypeptide chain comprising an amino acid sequence
3 defined by residues 16-123 of Seq. ID No. 3 (ALK-2), or an
4 OP1-binding analog thereof;
- 5 (ii) a polypeptide chain comprising an amino acid sequence
6 defined by residues 24-152 of Seq. ID No. 5 (ALK-3),, or an
7 OP1-binding analog thereof;
- 8 (iii) a polypeptide chain comprising an amino acid sequence
9 defined by residues 23-122 of Seq. ID No. 7 (ALK-6),, or an
10 OP1 binding analog thereof;
- 11 (iv) a polypeptide chain having binding affinity for OP-1 and
12 sharing at least 40% amino acid identity with residues 23-
13 122 of Seq. ID No. 7 (ALK-6),;
- 14 (v) a polypeptide chain having binding affinity for OP-1 and
15 encoded by a nucleic acid obtainable by amplification with
16 one or more primer sequences defined by Seq. ID Nos. 12-15;
17 or
- 18 (vi) a polypeptide chain having binding affinity for OP-1 and
19 encoded by a nucleic acid that hybridizes under stringent
20 conditions with a nucleic acid comprising the sequence
21 defined by nucleotides 256-552 of Seq. ID No. 7 (ALK-6),;
- 22 in a method for antagonizing
- 23 (i) OP-1 binding to a cell surface receptor; or
- 24 (ii) induction of an OP-1 mediated cellular response.
- 1 25. A method for antagonizing activin binding to a cell surface receptor,
2 the method comprising the step of:
- 3 providing a cell expressing a said receptor with a protein having
4 binding specificity for the amino acid sequence defined by
5 residues 16-123 of Seq ID No. 3 or an OP-1 binding sequence
6 variant thereof, said protein sharing at least 60% amino acid

- 7 sequence identity with residue 335-431 of the sequence defined by
8 Seq ID No. 9,
9 such that said protein, when provided to said cell, is competent
10 to interact specifically with said receptor, thereby
11 substantially inhibiting activin binding to said receptor.
- 1 26. A method for antagonizing BMP-4 binding to a cell surface receptor, the
2 method comprising the step of:
3 providing a cell expressing a said receptor with a protein having
4 binding specificity for the ligand binding domain defined by
5 residues 24-152 of Seq ID No. 5 (ALK-3), or residues 23-122 of
6 Seq ID No. 7 (ALK-6), or an OP-1 binding sequence variant
7 thereof, said protein sharing at least 60% amino acid sequence
8 identity with residues 335-431 of the sequence defined by Seq ID
9 No. 9,
10 such that said protein, when provided to said cell, is competent
11 to interact specifically with said receptor, thereby
12 substantially inhibiting BMP-4 binding to said receptor.
- 1 27. Use of the OP-1 receptor binding analog of claim 12-14 in the method of
2 claim 25 or 26.

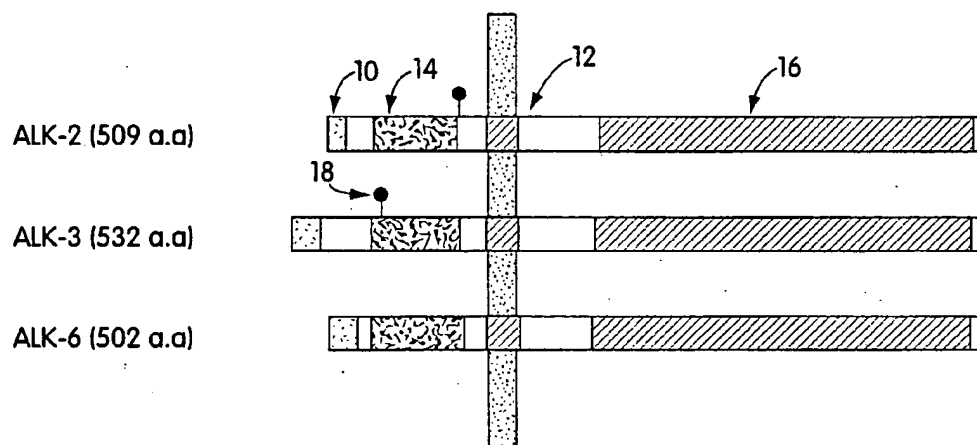


Fig. 1

2/3

-----LPSPSMEDEKPKVNPKLYMCVCEGLSCGNE---DHC	EQQCFS	SL	SIN	DGF	HVY	ALK-2
QGNLDSMLHGTGKMSDSDQKKSENGVTLAPEDTLPLFLKCYCSG-HCPDDA	INNTC	ITNG	HCFA	II	EEDD	QGETT
-----PTPRPKILRCKCHH-HCPEDSVNNICSTDG	YCFT	MI	EEDD	SC	MPV	ALK-3
-----PTPRPKILRCKCHH-HCPEDSVNNICSTDG	YCFT	MI	EEDD	SC	MPV	ALK-6
-----PTPRPKILRCKCHH-HCPEDSVNNICSTDG	YCFT	MI	EEDD	SC	MPV	ALK-2
-----PTPRPKILRCKCHH-HCPEDSVNNICSTDG	YCFT	MI	EEDD	SC	MPV	ALK-3
-----PTPRPKILRCKCHH-HCPEDSVNNICSTDG	YCFT	MI	EEDD	SC	MPV	ALK-6

Fig. 2

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-RKFKRRNQERLNPDPVEYGTIEGLITTNVGDSTLADLLDHSCTSGSGSLPFLVQRTVARQITLLECVGKGRYG	ALK-2
---KSISSRRRYNRDLEQD---EAFIP---VGES--LKDLIDQSQSSGSGSLPLLQRTIAKQIQMVRQVQKGRYG	ALK-3
FRYKRQEARPRYSIGLEQD---ETYP---PGES--LRDLIEQSQSSGSGSLPLLQRTIAKQIQMVRQIQKGRYG	ALK-6
----->	
EVWRGSMQGENVAVKIFSSRDEKSWFRETELNTVMLRHENILGFIAADMTSRHSSTQIMLITHYHEMGSLYDYL	ALK-2
EVWGMGKWRGEKVAVKVFFTTTEEASWFRETEIYQTVLMRHENILGFIAADIKGTGSWTQLYLITDYHENGSLYDFL	ALK-3
EVWGMGKWRGEKVAVKVFFTTTEEASWFRETEIYQTVLMRHENILGFIAADIKGTGSWTQLYLITDYHENGSLYDYL	ALK-4
QLTTLDTVSCIRIVLSIASGLAHLHIEIFGTQCKPAIAHRDLKSKNILLKKNQCCCIADLGLAVMHSQSTNQLDV	ALK-2
KCATLDTRALLLKLAYSACGLCHLHTEIYGTQCKPAIAHRDLKSKNILLKKNQSCCCIADLGLAVKFNSDTNEVDV	ALK-3
KSTTLDAKSMLKLAYS SVSGLCHLHTEIFSTQCKPAIAHRDLKSKNILLKKNQTCCCIADLGLAVKFIISDTNEVDI	ALK-6
----->	
GNNPRVGTKRYMAPEVLDETIQVDCFDSTYKRVDIWAFLGLVLWEVARRMVSNGIVEDYKPFYDVVPNDPSEEDMR	ALK-2
PLNTRVGTKRYMAPEVLDESINKNHFQPYINADIYSFGLIIWEMARRCITGGIVEEYQLPYNMVPSDPSYEDMR	ALK-3
PFNTRVGTKRYMPPEVLDESINRNHFQSYINADMYSFGLILLWEIARRCVSGGIVEEYQLPYHDLVPSPDPSYEDMR	ALK-6
----->	
KVVCVDQQRPNIPNRWFSDP TLTS LAKLMKECWYQNPSARLTALRIKKTLTKIDNSLDKLTDC	ALK-2
EVVCVKRLRPIVSNRWNSDECLPAVLKLMSECAWENPASRLTALRIKKTLAKMVESEQDVRI	ALK-3
EIVCMKKLRPSFPNRWSSDECLRQMGKLMTECAQNPASRLTALRVKKTLAKMSESEQDIKL	ALK-6
----->	

Fig. 3

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